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THE FILTRATION OF BACTERIA[1]

STUDIES IN BACTERIAL METABOLISM CIII

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INTRODUCTION

THE belief exists in some laboratories that bacteria, ordinarily deemed unfilterable, may under certain circumstances become so altered by chemical means, by cultivation for long periods of time in fluid media or through animal inoculation as to find their way through the pores of filters that would ordinarily restrain passage of the corresponding bacteria in their unfiltered state. The literature on this subject has become quite voluminous,[2] and opinion is now rather sharply divided into two groups; the "filtrationists," those who believe that bacteria may be filtered in some manner, and the "non-filtrationists," those who deny this possibility. This separation into two opposing camps is quite natural, because if one admits the possibility of filtration with one typical, ordinarily non-filterable microbe, one would rather logically be forced to admit that under suitable conditions all ordinary bacteria might be made filterable.

The problem of bacterial filterability has more than academic significance. There is a heterogeneous but formidable group of diseases of man and animals to which the term "filterable virus" is applied. The natural history of many of these "filterable virus" diseases—their clinical course, their method of dissemination and of inducing immunity—is generically akin to microbic diseases in which the etiological

[1] De Lamar Lecture, Johns Hopkins University School of Hygiene and Public Health, January 12, 1932.

[2] It is reviewed carefully by Klieneberger, "Bakterienpleomorphismus und Bakterienentwicklungsgänge," Erg. d. Hyg., Bakt., Immunitätsforsch. u. Exp. Therapie, Berlin, 11, 499–555. 1930.

agents are presumably known, and cultivable in appropriate media. Up to this time, however, the incitants of this "filterable virus" group, whatever they may be, have not for the most part been cultivated unequivocally outside their respective hosts upon artificial, laboratory nutritives.

It is surmised, and there is basis for this supposition, that the "viruses" of some of these diseases exist in the infected body in the filterable state, and it is not without some significance that many, if not most of these viruses enter, and probably leave the body, through the respiratory tract and its appendages. The possible import of this will be commented upon later. It is not proposed to discuss here the diseases allocated to the "filterable virus" group, but to present in detail some rather extensive studies upon the filterability of bacteria which may be relevant. These will be recounted at some length, as certain conclusions drawn from them would seem to indicate that the older division of microorganisms into "filterable viruses" and non-filterable bacteria may perhaps require some revision.

The experiments which led to the studies reported here have been described elsewhere in detail,[3] and will not be further referred to here, except for the statement that the first indication of the dual existence of an organism in a filterable and a non-filterable state was obtained from a study of blood from a small series of sporadic cases diagnosed clinically as influenza. A coccus was isolated from three of these cases in a special, protein-rich, peptone-poor medium (K medium);[4] at first in a non-visible, filterable state, and later apparently recovered after suitable procedure, as a visible, cultivable, non-filterable organism. From this wholly unexpected event, it was surmised that an actual, fundamental bacteriological principle was involved. If this be true, then other, well-established bacteria should also be rendered filterable experimentally, using the same general procedure, and recovered again in the non-filterable state.

Preparation of Culture

The organism chosen to test this premise was *B. typhosus*. The strain (Rawlin) was an old one, long in the laboratory. It has been used for many years in the customary manner for diagnostic purposes. It grows readily in plain, nutrient broth containing peptone and meat extractives. It also grows quite readily in the protein-rich, peptone-free K medium, prepared from hog intestine.[4] This is important. If the organism had failed to grow well in K medium, it would obviously have been less suited for these filtration experiments. The recital of an actual experiment with this strain of *B. typhosus* will make the procedure followed clear. Comments at the proper places will indicate the phenomena elicited. To insure purity in the usual bacteriological manner, this Rawlin strain of *B. typhosus* was replated three times upon agar. Between platings, growth from representative colonies was obtained in plain, nutrient agar. A colony from the last (third) plating was cultivated upon agar on October 29.

Filtration of Culture

Procedure

November 2. At 4 P. M. a generous loopful of this agar culture was introduced into 6 cc of K medium.[5] This was incubated at 37° C. over night.

November 3. Filtration I. At 10 A. M., growth being satisfactory, as estimated by a very distinct increase in turbidity above that of an uninoculated control, incubated in parallel, this 6 cc of K medium culture, after dilution with four volumes of sterile salt solution, was filtered through a Berkefeld "N" filter. (This filter did not pass a broth culture of *B. typhosus*). The vacuum used was less than 4 inches, water pressure, and the time of filtration was less than 10 minutes. The filtrate was clear. The filtrate was distributed at once, with sterile precautions, to the following media: to plain, nutrient broth; 1 cc, 1/2 cc, 1 drop; to K medium;[6] 1 cc, 1/2 cc, 1/4 cc, 1/10 cc, 1 drop. Incubation of these cultures together with uninoculated controls, was practiced at 37° C.

November 4. 10 A. M. Growth, as indicated by turbidity above parallel controls, occurred in all the tubes, both those in plain broth, and those in K medium. From the broth culture containing 1 drop of K medium filtrate, sugar fermentation reactions in glucose, lactose, saccharose and mannitol were made. After 24 hours, acid appeared in glucose and mannitol; saccharose and lactose were not acidified. An agar plate was also made. At the end of 24 hours, the colonies were typical and the culture appeared to be pure. Finally, after the sugar fermentation tests and agar plate had been started, a macroscopic agglutination test (dilution 1/500) was performed with this same broth culture. The culture agglutinated after 40 minutes' incubation at 37° C. An agar plate

[3] Patten Lecture, "Observations upon the Filterability of Bacteria, Including a Filterable Organism Obtained from Cases of Influenza," *Northwestern University Bulletin*, Vol. 32, No. 5, 1931; SCIENCE, 74, 129–139, 1931.

[4] "Mediums for the Isolation and Cultivation of Bacteria in the Filterable State," *Northwestern University Bulletin*, Vol. 32, No. 8, 1931.

[5] 100 mg. dried intestine, 6 cc Tyrode solution, containing neither glucose nor glycerin. This mixture is thoroughly shaken to wet the dried intestine powder, and then sterilized in the autoclave, 15 pounds for 15 minutes. *This medium should be slightly turbid when cool.*

[6] 6 cc, prepared as indicated in footnote 5, was used.

was also inoculated from the K medium tube which had received one drop of the K medium filtrate. The next day (November 5), typical colonies of *B. typhosus* were found in apparently pure culture. After transfer to broth, and proper incubation, they agglutinated with specific typhoid antiserum. Hence, from this first filtration, both the broth and the K medium tubes inoculated therefrom contained viable organisms which subsequently were identified as *B. typhosus* by plating, by fermentation reactions and by agglutination tests. The remainder of the tubes were reincubated for another 24 hours, together with their proper controls at 37° C.

November 5. Filtration II. 10 A. M. The tube of K medium which received 1/10 cc of the first filtrate (November 3), having a very noticeable turbidity after these 48 hours of incubation, was diluted with 4 volumes of salt solution and filtered through a Berkefeld "N" filter. The vacuum used was less than 4 inches, on the water-gauge, and the time required was somewhat less than 10 minutes. This filtrate was distributed as follows: to plain broth; 1 cc, 1/2 cc, 1 drop: to K medium; 1 cc, 1/2 cc, 1/4 cc, 1/10 cc, 1 drop. Incubation was practiced as before at 37° C.

November 6. 10 A. M. All cultures made November 5 grew; the plain broth cultures were distinctly less turbid than the corresponding ones of November 4. The K medium cultures, on the contrary, were rather more cloudy than the corresponding ones of November 4. Fermentation reactions were set up from the plain broth culture (that one inoculated with 1 drop of K medium filtrate of November 5); also an agar plate was inoculated. On November 7 the fermentation reactions were typical. The colonies on the agar plate were somewhat mucoid in appearance. An agglutination test (macroscopic, 1/500) made upon this broth culture was positive in 90 minutes. It will be recalled that the corresponding agglutination test made upon the broth culture recovered from the first filtration (November 3) was complete in 40 minutes. The growth in K medium failed to agglutinate in typical fashion, but a broth culture made from a K medium growth agglutinated characteristically. The significance of this observation, which has been repeated several times, is yet to be elucidated. From the results of this second filtration, it appears that there is some slight tendency both toward a change in the appearance of the colonies developing upon agar from broth inocula, and also a distinct tendency toward slowing of the speed of agglutination. Nevertheless, it is adduced that this second serial filtration of *B. typhosus*, and its recovery in the non-filterable state, was successfully accomplished.

November 6. 11 A. M. *Filtration III.* The K medium culture of November 5, containing one drop of filtrate, was diluted with 4 volumes of salt solution and filtered, this time through a Berkefeld "W" filter. The vacuum used was less than 5 inches on the water-gauge, and the time required was about 15 minutes. The filtrate was distributed as described above, in the following media: to plain broth; 1 cc, 1/2 cc, 1 drop: to K medium; 1 cc, 1/2 cc, 1/4 cc, 1/10 cc, 1 drop. Incubation was practiced at 37° C.

November 7. 9:30 A. M. The cultures in K medium all showed increased clouding above the control tube, which had been incubated in similar fashion. Of the plain broth tubes, on the contrary, none grew; at least there was no discernible turbidity at this time. Incubation of all the cultures was again practiced at 37° C.

November 9. Noon. The K cultures of November 6 had increased very distinctly in turbidity, and the broth culture of November 6, containing 1 cc of filtrate, had developed a faint but distinct turbidity. An agglutination test performed upon this broth culture, after inoculation of sugar fermentation tubes and agar plates, was partly clumped after 2 hours, and completely clumped after 18 hours in the ice-box. Agglutination of the cultures in K medium was negative. The colonies upon agar plates were rather mucoid in appearance. Sugar fermentation reactions were typical, but rather slow in developing. Recovery of the organism in its non-filterable state after each of these three several filtrations was achieved. The identification was made in each instance from growth induced in nutrient broth, by the appearance of colonies upon agar plates (test for purity), by fermentation reactions, and by agglutination (1/500) with specific antityphoid serum.

Each of the cultures made in K medium on November 6 was inoculated into plain broth, but even after 10 days' incubation, visible growth did not occur. It is assumed therefore that this serial, prolonged cultivation of *B. typhosus* in the protein (K) medium has resulted in an acclimatization of the organism in its filterable state to the protein medium, with a concomitant loss of accommodation to the simpler (peptone, meat extractives) medium. It has, in other words, become "proteophilic" instead of "peptophilic." It is surmised that a principal function of this filtration through the Berkefeld "N" and "W" filters is to strain out, and hold back, the non-filterable forms of the organism, passing those forms which not only are indeed filterable but also which have become more and more acclimatized to the protein environment. The final result of these three serial filtrations is apparently to establish culturally this filterable, protein-cultiviable form of the organism, which is reluctant to grow in ordinary media. To

answer the obvious inquiry at this point: recovery of the bacillary form was eventually achieved through inoculation of this filterable state of *B. typhosus* upon plain, nutrient agar, after incubation under partially anaerobic conditions for several days. The details will appear later. For the present, the point at issue is the premise that *B. typhosus* is, or may be, filterable if it is cultivated for several transfers in a "protein" (K) medium. Also, that it may be recovered in the non-filterable (peptophilic) form as above indicated.[7]

The subsequent history of this thrice filtered culture of *B. typhosus* and its maintenance in the filterable state is as follows: the tube of K medium inoculated November 6, containing one drop of filtrate of that date, was used. Transfers were made to fresh K medium November 9 and November 12, respectively, with incubation at 37° C. between times. The next transfer was made in California, November 16, and daily thereafter through November 22. With the exception of November 13, 14 and 15, during which time the culture was in transit, incubation of the subcultures was practiced at 37° C. From November 22 to November 28, the organism was again in transit, but reinoculation and reincubation were resumed on this date. On January 4 the strain is still alive. It still grows in K medium, but not at all in nutrient broth. It has retained its proteophilic properties, and apparently has not regained its original peptophilic state, as manifested by lack of visible growth in nutrient broth, even after several days' incubation. Thus, so it appears, a viable organism in the filterable state, incapable of visible growth in plain, nutrient broth, has been obtained by direct descent in culture through serial growth in an artificial, sterilizable medium (K medium) from an authentic, typical, plain nutrient broth culture of *B. typhosus*. It is left to the microbic pragmatists and dialecticians to debate whether or not this viable, cultivable, filterable organism thus obtained fulfils any, some or all of the criteria of a "filterable virus."

Summary

This detailed protocol has been recited in considerable length because it contains what appears to be unequivocal evidence that a strain (Rawlin) of *B. typhosus* has been filtered not once, but actually thrice serially through Berkefeld filters, the first two filtrations through Berkefelds of N porosity, the third filtration through a Berkefeld of the W porosity, making three filtrations in all in a period of four days. These filtrations were performed on November 3, 5 and 6, respectively. Between filtrations, and prior to each of them, growth was elicited in K medium. The organisms developing after the first, the second and the third filtrations (November 3, 5 and 6) were recovered successively in the non-filterable state in nutrient broth and tested for their identity and purity by sugar reactions, by agglutination with specific typhoid serum and by plating upon agar. These tests were performed not only with the cultures of filtrates in plain nutrient broth, but also with subcultures made from K medium, in plain nutrient broth, with the exception of the K culture from the third filtration. This failed to grow in nutrient broth, although it grew well in successive K medium transfers.

Concerning the proportion of filterable to non-filterable forms, no definite information is available. However, inasmuch as the cultures were diluted with salt solution to 1/5 their original concentration prior to filtration, and inasmuch as 1 drop and 1/10 cc, respectively, of the diluted filtrates gave growths that were subsequently identified as *B. typhosus*, by fermentation reactions, by agglutination and by plating, it may be assumed that at least 1/50 cc of the unfiltered cultures in K medium contained viable typhoid microbes in the filterable state.

Comment

Imperfect filters: The old familiar contention that the filters which were used may have leaked, must be resurrected at this point. The theoretical possibility of leaky filters must always be admitted, and, except for two significant facts, it should be freely admitted in these experiments. Overlooking for the moment the fact that a filterable organism, incapable of growing in nutrient broth, was obtained through filtration, as above indicated, attention is directed to the fact that the filters used in these studies had been tested previously for passage of broth cultures of *B. typhosus* with negative results; hence it is assumed that the organisms do actually become filterable under the conditions specified, although the possibility still remains that the porosity of these tested filters may have changed in the interim. Also, and this may be significant, the broth culture made from the filtrate in broth (1 cc) of November 5, likewise failed to pass through one of the "N" filters used for these experiments. It is rather generally believed that Berkefeld "W" filters are too fine to pass bacteria. Attention is directed at this point to Bechhold's[8] important con-

[7] It is pertinent to inject here the rather obvious comment that the time relations met with in filtration experiments vary not only with the kind of organism, but also with the strain itself. Thus, one typhoid strain studied became "proteophilic" after one filtration; and another strain required four filtrations at two day intervals to become "proteophilic."

[8] H. Bechhold, "Porengrosse von Bakterienfiltern und Seibwirkung," *Zeit. f. Hyg. u. Infektskr.*, 112, 3 Heft, 1931.

tribution to relations between size of pores in filters (*cf.*, Berkefeld N and W, p. 416) and their capacity to withhold bacteria under definite conditions.

The other rejoinder to the plea of leaky filters, and, coincidentally perhaps the most suggestive new idea developed in these experiments, aside from the filterability and recovery of the non-filterable form of *B. typhosus*, is the gradual weaning of the filterable form of *B. typhosus* from its original (peptophilic) state, in which form it is non-filterable, to the filterable (proteophilic)[9] state, in which form it appears eventually to pass quite readily through even the finest Berkefeld (W) filter. In the peptophilic state, the organism grows readily in nutrient broth. In the fully established filterable state, achieved only by a sufficient number of serial filtrations to eliminate the peptophilic organisms, the microbe fails to grow in nutrient broth. It grows very well, however, in K medium.

It is, therefore, apparently a simple, rather rapid procedure to induce the filterable state by inoculation into K medium. Once the organism, freed from non-filterable tendencies by repeated filtration with inter-cultivation in protein (K) medium, has become accustomed, or better acclimatized, to the protein pabulum, it becomes increasingly difficult, at least in these experiments, to reinduce the non-filterable state.

At this time, an unexpected experience in the filtration of a culture of *B. coli* seems pertinent, in that it illustrates some of the pitfalls associated with the phenomena of microbic filtration. This particular strain of coli was isolated in 1909, and has been in laboratory stock ever since. The first attempt at filtration resulted in recovery of a small, yellow coccus. The yellow coccus was devoid of glucose fermenting power. Repeated platings of the original culture upon agar failed to reveal this coccus. Finally, resort was made to cultivation in K medium for 30 hours prior to plating. Then about 2 per cent. of the colonies on agar were of this yellow coccus, the remainder being typical coli. The K medium had been shown to be sterile prior to inoculation. Filtration of the purified colon bacilli, had by plating from K medium, was accomplished in good form, and the organism was filtered three times in five days with intervening growth in K medium. Between filtrations, typical *B. coli* were recovered and identified by fermentation reactions. For comparison, another stock culture of *B. coli* was examined. It failed either by direct plating, by plating after growth in K medium, or upon filtration, to reveal this yellow coccus. The possibilities enmeshed in this experience with the yellow coccus are many; space forbids more than mention of the facts as they were observed at this time.

[9] Throughout this discussion, the terms "peptophilic" and "proteophilic" are used symbolically to suggest rather definitely a chemical difference between peptone on the one hand, and the more or less unaltered protein constituents of dried intestine on the other hand.

Morphological Changes during Filtration

Returning to the filtration of *B. typhosus*, the question very naturally arises, Are there changes discernible in the typhoid bacilli during the process of inducing the filterable state that may actually be seen under the microscope? One rather suggestive difference between the appearance of the first growth of *B. typhosus* in K medium immediately prior to filtration, and of the organism after filtration, is the relative abundance of bacillary forms, with and without granules within their substance, in the former, as shown by staining with old methylene blue, by examination with the 1/12 immersion lens directly and by dark-field illumination. This is sharply in contrast to the apparent absence of these bacillary forms, with and without granules, in the filtrate immediately after filtration. In both the unfiltered primary growth in K medium and in the filtrate of this primary K medium culture, however, there are numerous granules. Some of these granules are inherent in the medium.[10] They are bluish or greenish yellow under dark-field illumination, and usually exhibit Brownian movement. In addition, in both filtered and non-filtered K medium cultures of *B. typhosus*, there are usually small, somewhat motile granules. These are, in fresh culture in K medium, often nearly as active in their motion as typhoid bacilli themselves. It must not be assumed from this observation that the filterable forms of all bacteria will exhibit true motility, however.

In the filtered medium, these granules and not the bacillary forms, appear to be the only motile bodies. Thus far, they have not been definitely stained, even by Giemsa or silver stains, and their identification therefore depends rather upon inference than upon rigorous proof.

It is necessary to reemphasize here that repeated filtration of cultures of *B. typhosus*, with interspersed cultivation in K medium, is required to eliminate the tendency of the filterable forms to go back to the bacillary form. As acclimatization progresses, with repeated filtration for the purpose of the elimination of the non-filterable forms, a time comes, usually after two or more successive filtrations at appropriate intervals, when the filterable (proteophilic) forms no longer respond to the "peptone urge" and remain

[10] It will be found that cultures grown in broth usually contain at least some bacilli with enclosed granules, and all media containing protein ingredients will contain at least some free granules. These, in uninoculated media, are non-motile, although they usually exhibit Brownian movement.

viable, but filterable from culture to culture in K medium. Such "proteophilic" organisms, in the filterable state, are difficult indeed to reestablish in the non-filterable condition. A condition similar to this may be readily conceived of as existing in the tissues of the animal or human body, where invading bacteria, originally non-filterable, exposed for periods of time to the tissue protein in absence of peptones, may thus become proteophilic, parasitized upon the proteins of the body, as it were, and difficult to recultivate in peptone media. Emphasis is laid at this point upon the biological significance of inducing this filterable state through cultivation of bacteria in media rich in nearly unaltered protein. In this respect, the experiments recorded above differ sharply from previous procedures in which strontium salts, or very old peptone broth cultures are relied upon to induce filterability.

Thus far, emphasis has been laid upon actual experiments. It has been found that *B. typhosus*, under cultivation in a protein-rich, peptone-poor medium passes rather rapidly and readily to the filterable state. While it can not be dogmatically denied that leaky filters permit of this passage, the fact that five different strains of *B. typhosus* (to restrict discussion to this organism for the present) have been thus filtered and recultivated, using some 24 different filters of the Berkefeld type, each time employing the finest pored filter (W) for the final separation of non-filterable forms, would appear to be reasonable evidence of the accuracy of the assertion. The part played by filtration in these experiments is merely passive. It removes those filterable, lineal descendants of the original stock culture which still manifest a tendency toward reversion into typical, non-filterable bacilli, under the nutritional stimulus of peptone, from the filterable forms, which have developed, so to speak, proteophilic propensities.

Relation of the Filterable State to Infection

Some interesting correlative information arises from this nutritional concept of the development of filterable forms of bacteria from non-filterable forms in the body itself, especially in relation to microbic infection.

Microbic infection, generally speaking, may be considered as actually taking place when the prospective invading organism passes through the barriers which usually suffice to keep it out, and actually penetrates into the protein fastness itself. A majority of bacteria, and presumably a majority of "viruses," gain entrance to the underlying tissues through epithelia, principally those of the intestinal and the respiratory tracts. A significant chemical difference between these two tracts should be emphasized here. The intestinal mucosa is almost continually bathed, on the epithelial side, in a medium rich in protein digestive products, which pass successively from the complex peptones and albumoses to simple peptids and amino acids. The latter, according to current information, are normally absorbed from the alimentary canal through the villi, and pass to the blood stream. Hence the mucosa of the intestinal tract is in a peptone environment, using the term "peptone" symbolically merely to indicate protein in various stages of digestion. The nutritional value of this medium is reflected in the luxuriance of the intestinal flora: some thirty trillions of visible and stainable bacteria are said to be eliminated each day in the fecal mass of a normal adult enjoying a normal diet. The respiratory tract, on the contrary, is a relatively sterile tract. Protein degradation products, except at those times when purulent bacteria are at work, are apparently absent. The significance of mucus in this connection can not be answered in light of available information. Stated somewhat differently, the digestive tract is proteolytic; the respiratory tract is aproteolytic. Bacteria that enter the digestive tract find abundance of protein digestion products available for their nutrition: bacteria that enter the respiratory tract do not normally find protein digestion products available for their nutrition. It would seem to follow that bacteria within the intestinal tract are in an environment that should tend to encourage their existence in a non-filterable (peptophilic) state. Bacteria that gain entrance to the respiratory tract would appear to be in an environment that normally should tend to encourage their existence in a filterable (proteophilic) state.[11] In light of this rather striking difference between the two tracts, it is not without significance that many if indeed not most of the contagious so-called "filterable viruses" according to current information appear to enter, and to leave, the body through the respiratory rather than the intestinal path.

Microorganisms that pass from the respiratory tube actually into the tissue of the lung, whether they are initially filterable or not, become confronted with a protein-rich, peptone-poor medium, as they penetrate the epithelia of the respiratory tract. From what has been stated above, this is one condition which tends to induce and to perpetuate the filterable state. Similarly, upon passing back from lung tissue to the respiratory tube, the same condition apparently prevails unless there is pus formation. Pus, as is well known, contains products of protein digestion. It would seem to follow logically that bacteria in the filterable state should, theoretically at least, be not

[11] The pneumococcus, a frequent incitant of pneumonia, is habitually a purulent organism. Pus is said to be rich in protein degradation products.

uncommon in non-purulent infections of the respiratory tract. Bacteria leaving the body from the intestinal tract, on the contrary, are exposed to nutritive conditions conducive to the non-filterable rather than the filterable state. Hence it is perhaps not necessary to reiterate that many, if not most, of the "filterable viruses" have been found thus far in association with the respiratory, rather than the intestinal tract. This is not to be construed as an assumption that all "filterable viruses" exist in a non-filterable as well as a filterable state: only precise experiments with each disease entity will determine just what the limits to be applied ultimately to the term "filterable virus" shall be: nor is it by any means the whole story. *B. coli,* like *B. typhosus,* seems to become filterable without much difficulty by cultivation in the proper manner in protein media, but the fact that this microbe may thus become filterable does not explain why it is not ordinarily an invader of the body. The nature of the weapons with which certain kinds of bacteria, and not others, may force entrance through the epithelia which ordinarily suffice to keep microbes out, is yet to be determined. Just what part the filterable state of bacteria may play in the vastly complex phenomena of infection and immunity remains to be revealed. And there are exceptions to this hypothesis of association between protein nutrition and the filterable state. Leprosy appears to be such a case. Leprosy bacilli are readily stained within the tissues of lepers; that is, they exist there in a presumably non-filterable state. There is little evidence that there is much, if indeed any, tissue digestion round about them. Nevertheless, exceptions to the contrary, there is after all apparently a very distinct general biological parallelism between the occurrence of non-filterable, stainable bacteria, growing in ordinary media under the nutritional stimulus of peptone (peptophilic state) and filterable, not stainable organisms growing in protein-rich, peptone-poor media under the nutritional stimulus of protein (proteophilic state). And the well-established difficulties surrounding the isolation of microbes from some of the so-called "filterable viruses" (which appear to be developing in the protein tissues of the body and are refractory to cultivation in peptone media, even enriched with tissue) have their nearly precise counterpart in this connection with the corresponding experimental difficulties encountered in cultivating the fully protein acclimatized, filterable form of *B. typhosus* in peptone media, even those enriched with blood. It would appear indeed that one rather striking feature of the experiments with filterable forms of the typhoid bacillus is this very establishment of the proteophylic state, refractory to cultivation in ordinary, or enriched peptone media. If this indeed be the case, then it might be rather confidently predicted that at least some of the infections refractory to artificial cultivation should be approachable from the use of suitable protein media. A word about K medium should be injected at this point: it is very crude. This has been emphasized again[3] and again.[4] There is no more reason, *a priori,* for expecting successful isolation of organisms from influenza, common cold and smallpox, to mention three possible sources of culture, in K medium in its present crude state, than there is in expecting successful isolation of *Tr. pallidum,* meningococcus and tubercle bacillus, using merely plain, nutrient broth. Special modifications, to meet the needs of specific organisms, both for isolation and for cultivation of specific organisms in a filterable state, must be applied to the medium, as is required of the usual laboratory media for similar reasons. This is well exemplified by the recent work of Mellon,[12] who has just reported the successful filtration of the tubercle bacillus, and its recovery in the non-filterable state, using a modified K medium.

Conclusions

(1) The intimate details of three successive filtrations of an authentic strain of *B. typhosus,* performed in four days, are recorded.

(2) The details of recovery of *B. typhosus* in the non-filterable state, and its identification by colony formation, by fermentation reactions, and by agglutination tests, are stressed.

(3) Attention is drawn to the perpetuation of *B. typhosus* in the filterable state by cultivation in a protein [K] medium.

(4) Emphasis is laid upon the biological significance of proteins in inducing the filterable state in bacteria.

(5) Certain theoretical relationships are suggested between this artificially induced filterable state of bacteria and certain microbic infections of man.

OBITUARY

MEMORIALS

The section on medical history of the College of Physicians of Philadelphia, as reported in the *Journal* of the American Medical Association, held its stated meeting on March 14, in cooperation with the Henry Phipps Institute, the Philadelphia Health Council and Tuberculosis Committee and the Philadelphia Association of Tuberculosis Clinics to commemorate the

[12] Mellon, *Proc. Soc. Exper. Biol. and Med.,* Vol. 29, No. 2, p. 206, 1931.

fiftieth anniversary of the discovery by Robert Koch, March 24, 1882, of the tubercle bacillus. Addresses were made by Drs. Henry R. M. Landis, on "Reception of Koch's Announcement in the United States"; Theobald Smith, Princeton, N. J., "Koch's Views on the Stability of Species among Bacteria"; Lawrason Brown, Saranac Lake, New York, "What Koch Meant to Tuberculosis," and Damaso de Rivas, "Personal Reminiscences of Robert Koch."

CONTRIBUTIONS are invited to a memorial to Sir Andrew Balfour, first director of the London School of Hygiene and Tropical Medicine, who died a year ago. The medical men supporting the appeal include: Sir James Crichton-Browne; Sir Matthew Fell, late Director-General A.M.S.; Professor W. W. Jameson, dean of the London School of Hygiene and Tropical Medicine; Colonel P. S. Lelean, professor of public health, Edinburgh University; Dr. A. T. Stanton, chief medical adviser to the Secretary of State for the Colonies; and Dr. C. M. Wenyon, director-in-chief, Wellcome Bureau of Scientific Research.

A DINNER of the Royal College of Surgeons, at which the Lord Mayor of London was a guest, was held on February 11 to commemorate the two hundred and fourth anniversary of the birth of John Hunter. Dr. David Ross, the president, was in the chair. The toast "The Memory of John Hunter" was honored in silence. The president said that the presidential chair in which he sat would eventually be presented to the Royal College of Surgeons. He recounted the activities of the society during the past year and concluded by presenting Dr. Griffith Ifor Evans, of Carnarvon, with the Hunterian Medal, the first struck in gold, for an essay on "Chronic Familial Syphilis." The medal, he said, has been designed by Mr. W. Thornton Shiells, and a plaque was presented by the honorable treasurer, Dr. Irwin Moore.

RECENT DEATHS

HARVEY M. HALL, staff member of the division of plant biology of the Carnegie Institution of Washington, a student of botany and ecology, died on March 11 at the age of fifty-eight years.

A CORRESPODENT writes: "Dr. Charles Ford Langworthy died in Washington on March 3 at the age of sixty-seven years. Dr. Langworthy was a chemist and was for many years one of the foremost workers in America on nutrition problems. During most of his active life he was connected with the U. S Department of Agriculture, first as associate editor of the *Experiment Station Record* and later as chief of nutrition investigations and chief of the Office of Home Economics."

MARY FRANCES SEYMOUR, professor of biology in Catawba College, Salisbury, N. C., since 1925, died on March 2. Professor Seymour was associate professor of biology at the North Carolina College for Women, Greensboro, N. C., 1916–1923.

A CORRESPONDENT writes: "Professor A. A. Jaczewski, eminent Russian mycologist and plant pathologist, died in Leningrad on February 12. Dr. Jaczewski organized and was director of the Jaczewski Institute of Mycology and Plant Pathology, which was, in this field, the central institute of all Russia. Dr. Jaczewski visited the United States in the summer of 1921. He was well and widely known for his texts and other scientific contributions. In the field of systematic mycology and plant pathology he has long been recognized as the outstanding research leader and teacher of Russia."

SCIENTIFIC EVENTS

PSITTACOSIS IN CALIFORNIA

ON the basis of recent deaths from psittacosis in California, and positive findings in parrakeets from various parts of the state made by Dr. K. F. Meyer, of the Hooper Foundation for Medical Research of the University of California, the State Department of Public Health has adopted new rulings concerning the handling of parrakeets.

One of the new regulations makes psittacosis a reportable disease, and another restricts the importation and exportation of love birds and birds of the parrot family. The latter regulation reads in part as follows:

Whereas, It has been determined that birds belonging to the parrot family and infected with disease, and love birds so infected have infected other birds of the same family and that some of said birds have been infected with disease which may be transmitted to man; and

Whereas, It has become necessary to take action to prevent further infection of such birds in California and thereby prevent the extension of the disease to human beings; therefore, be it

Resolved, by virtue of the authority vested in this board by section 2979 of the Political Code of California, that the importation into and the exportation from the State of California of all birds of the parrot family and love birds, be prohibited for a period of not to exceed six months; provided that shipments of such birds from a foreign country consigned to another state or consigned from another state to a foreign country or consignments of such birds between other states, may be

permitted to pass through California if under official seal.

The *Weekly Bulletin* for February 20, of the State Department of Public Health says: "A brief summary of the psittacosis situation in California during the past few months reveals the appearance of twelve cases and six deaths. These patients had in every instance except one been in contact with recently purchased parrakeets, most of which were purchased from itinerant bird venders.

"The laboratory work in connection with these cases was done by Dr. F. K. Meyer at the Hooper Foundation for Medical Research. Positive findings were reported in parrakeets from Grass Valley, San Luis Obispo, Woodland and San Francisco. Not all of these parrakeets had been associated with cases in human beings. Positive results were obtained also from autopsy specimens from one of the patients who died of psittacosis."

GENETICS SOCIETY OF AMERICA

AT the recent New Orleans meetings, the genetics sections of the American Society of Zoologists and the Botanical Society of America were reorganized as the Genetics Society of America. The group known as the Geneticists Interested in Agriculture was discontinued with the understanding that symposia of interest to agriculturists will be held by the new society. Dr. R. A. Brink was chosen to cooperate with the society officers in organizing this part of the program.

The executive committee of the society consists of the following officers and past chairmen: L. C. Dunn, *president;* F. D. Richey, *vice-president;* P. W. Whiting, *secretary-treasurer;* L. J. Stadler, *past chairman;* L. J. Cole, *past chairman.* The society now consists of 289 members of whom 153 are also members of the American Society of Zoologists and 90 are members of the Botanical Society of America.

The officers of the society urge members to suggest to any of their acquaintances who are interested in any field of genetics to apply for membership. Names endorsed by two members may be sent to the secretary at any time. Teachers of genetics, advanced graduate students, and research assistants who may be interested in attending the meetings or in receiving copies of abstracts giving the latest results of investigations are welcomed to membership. It is particularly urged that Canadians or others who may be so situated as to be able to attend the meetings will not hesitate to apply for membership.

It is expected that the meetings will be conducted very similarly to those that have been previously held by the genetics sections. The executive committee will, however, welcome suggestions from members in reference to program rules or any other affairs of the society. All suggestions mailed to the secretary will be circulated among the other members of the Executive Committee for their consideration.

Copies of the constitution and by-laws will be sent upon request. Annual dues are $1.00. Copies of programs and abstracts of papers to be presented at the annual meeting will be furnished without charge to members in good standing.

P. W. WHITING,
Secretary-Treasurer

DEPARTMENT OF ZOOLOGY,
UNIVERSITY OF PITTSBURGH,
PITTSBURGH, PA.

THE SUMMER SESSION OF THE CHEMISTRY DEPARTMENT OF THE JOHNS HOPKINS UNIVERSITY

AN experiment in graduate work is to be initiated in the chemistry department of the Johns Hopkins University during the summer session of 1932, in order to provide a unique opportunity for teaching and industrial chemists to confer with authorities of national and international repute on "Recent Developments in Chemistry." The work is planned as a series of conferences covering a period of five weeks, each week to be devoted to a particular phase of chemistry. This special course is in addition to the regular theoretical and laboratory courses in general, physical, organic and analytical chemistry, and arrangements have been made for those on limited leave to register for only one week, if so desired. In addition to the regular registration fee of three dollars, a nominal fee of five dollars per week will be charged. The complete series may be taken as a graduate credit course, without additional charge to students regularly registered in the summer session.

The conferences on "Recent Developments in Chemistry" will be directed by members of the graduate faculty whose work will be supplemented by invited lecturers. The program is as follows:

Program

June 27 to July 1: "Raman Effect and Problems in Molecular Structure," directed by D. H. Andrews. The week will close with a general discussion grouped around papers by the invited lecturers—Harold C. Urey, Oliver R. Wulf, John R. Bates, Henry Eyring and H. M. Smallwood.

July 5 to 9: "Colloidal Chemistry," directed by W. A. Patrick. A special lecture will be given on "The Colloidal Behavior of High Polymers," by Elmer O. Kraemer.

July 11 to 15: "Catalysis," directed by J. C. W. Frazer.

July 11 and 12: J. C. W. Frazer, "Structure of Catalysts; State of Adsorbed Molecules; Mixed Catalysts; and Poisoning of Catalysts."

July 13: A. B. F. Duncan, "Absorption of Gases or

Solids; Form of Adsorption Curves; Heat of Adsorption; and Activation Energy of Adsorption and Rate of Adsorption."

July 14: Louis S. Kassel, "Heterogenous Reactions."

July 15: Paul H. Emmett, "Studies on the Mechanism of Ammonia Synthesis Over Iron Catalysts."

July 15: A. T. Larson, "The Methanol Synthesis."

July 18 to 29: "Relation of Properties to Constitution of Organic Compounds," directed by E. Emmet Reid.

THEORETICAL

July 18 to 22: "The Relation of Structure to Physical Properties, Melting and Boiling Points, Optical Activity, etc., and to Chemical Properties, such as Reactivity."

July 18: E. Emmet Reid, "Introduction to Week's Work."

July 19: E. Emmet Reid, assisted by F. O. Rice's students, "Free Radicals."

July 20: M. S. Kharasch, "The Use of the Electronic Theory in Elucidating Reactions in Organic Chemistry."

July 21: P. A. Levene, "Chemical Structure and Optical Activity."

July 22: C. S. Hudson, "An Extension of Emil Fischer's Proof of the Configurations of the Sugars."

APPLICATIONS

July 25 to 29: "The Designing of Organic Compounds for Specified Purposes as Illustrated in Chemotherapy and in the Arts."

July 25: E. Emmet Reid, "Introduction to Week's Work."

July 26: David I. Macht, "The Present Status of Possibilities and Limitations in Regard to the Relationship between Chemical Structure and Physiological Action."

July 27: H. J. Barrett, "Relation of Resin Formation to Structure."

July 28: H. A. Lubs, "Relation of Color to Constitution in the Thioindigoid Dyes."

July 29: R. E. Rose, "The Relation of Structure to the Color of Dyes."

OTHER FEATURES

Another course of interest to chemistry teachers will be that in "Undergraduate Curriculum Content." This will be worked out to develop an improved curriculum for undergraduate chemistry students and will be conducted to some extent in connection with the conferences on "Recent Developments in Chemistry." Attendance at the latter will not be required, but will be encouraged as providing an opportunity for teachers to select at first hand and to organize material from up-to-date developments. Registrants in the course on "History of American Chemistry" will have the privilege of conferring with Dr. C. A. Browne, authority in the field of historical chemistry.

The following sound films will be presented as public lectures during the summer:

June 30: "Cosmic Rays," by R. A. Millikan.

July 14: "Oil Films on Water," by Irving Langmuir.

July 28: "Some Biochemical, Pharmacological and Medical Experiences as Told to Chemists," by J. J. Abel.

MEMBERS ELECTED BY THE WASHINGTON ACADEMY OF SCIENCES

THE following have recently been elected to membership in the Washington Academy of Sciences:

HONORARY MEMBER

Sir James Hopwood Jeans has been made an Honorary Member in recognition of his contributions to the dynamical theory of gases, to cosmogony, and to astrophysics. His brilliant applications of mathematical physics to the problems of astronomy have made him one of the leaders in the recent great advance in that science. Among his important publications are the following books: "The Dynamical Theory of Gases," "Problems in Cosmogony and Stellar Dynamics," and "Astronomy and Cosmogony." He is a research associate of the Carnegie Institution of Washington.

MEMBERS

Dr. Frederick Sumner Brackett, director of the division of radiation and organisms, of the Smithsonian Institution. Dr. Brackett is well known for his investigations in spectroscopy, including the development of thermopiles, and for his researches on plants and radiation, the results of which have been published in various journals.

Members newly elected to the Washington Academy of Sciences are characterized in the *Journal* of the academy as follows:

Dr. Robert Herman Bogue, research director, Portland Cement Association Fellowship at the Bureau of Standards. Dr. Bogue was elected to membership in recognition of his contributions to colloid chemistry and to the physical chemistry of silicates. He is the author of numerous papers on these subjects.

Professor Oakes Ames, professor of botany, supervisor of Biological Laboratory and Botanical Garden (Cuba), Arnold Arboretum and Botanical Museum, Harvard. Professor Ames was elected to membership in recognition of his contributions to systematic orchidology. He is the preeminent authority in this large and exceedingly difficult group of plants.

Dr. Thomas Barbour, director of the Museum of Comparative Zoology. Dr. Barbour was elected to membership in recognition of his contributions to herpetology and ornithology.

Dr. Johannes Hadeln Bruun, research associate at the Bureau of Standards. Dr. Bruun was elected to membership in recognition of his work on the separation and identification of the constituents of petro-

leum, the results of which have been published in various journals.

Charles Allen Cary, physiological chemist, Research Laboratories, Bureau of Dairy Industry. Mr. Cary was elected to membership in recognition of his contributions to the knowledge of nutrition and particularly the protein metabolism of milking cows. He is the author of numerous papers on these subjects.

Henry B. Collins, Jr., assistant curator, Division of Ethnology, U. S. National Museum. His election to membership was in recognition of his archeological researches in the southeastern section of the United States and in Alaska, and his contributions to physical anthropology.

Dr. James Fitton Couch, chemist, Bureau of Animal Industry. Dr. Couch was elected to membership in recognition of his work on the active principles of stock-poisoning plants. The results of his work have been published in various journals and bulletins.

Dr. Carl S. Cragoe, physicist, Bureau of Standards. Dr. Cragoe was elected to membership in recognition of his work on the thermodynamic properties of ammonia and of petroleum products.

Dr. Leon Francis Curtiss, physicist, Bureau of Standards. Dr. Curtiss was elected to membership in recognition of his investigations in radioactivity and cosmic radiation.

Dr. Francis Marion Defandorf, physicist, Bureau of Standards. Dr. Defandorf was elected to membership in recognition of his contributions to the science of electrical measurements, particularly in the field of high voltage.

Herbert N. Eaton, acting chief of the hydraulic laboratory, Bureau of Standards. Mr. Eaton was elected to membership in recognition of his work in aeronautics and hydraulics. He has written numerous articles on aeronautic instruments.

PRESENTATION TO PROFESSOR CONANT

THE William H. Nichols Medal of the New York section of the American Chemical Society for 1932 was presented on March 1 to Professor James Bryant Conant, chairman of the division of chemistry at Harvard University, in recognition of his work in organic chemistry, particularly in the chemistry of chlorophyll.

Professor Arthur E. Hill, of New York University, made the presentation. Other speakers were Professor James F. Norris, of the Massachusetts Institute of Technology, who discussed Professor Conant's personal career, and Professor Hans T. Clarke, of the College of Physicians and Surgeons, Columbia University, who recounted his scientific accomplishments. Mr. Walter S. Landis, chairman of the New York Section of the American Chemical Society, presided.

The Nichols Medal, established in 1903, is one of the most distinguished honors in American chemical science. The award, made for the research published during the past year, which in the opinion of the jury is most original and stimulative to further research, was bestowed on Professor Conant for his work in organic chemistry, particularly in the chemistry of chlorophyll. The late Dr. Nichols, the donor, was chairman of the board of the Allied Chemical and Dye Corporation and a charter member of the American Chemical Society.

Past winners include Professor William Lloyd Evans, of the Ohio State University, who received it in 1929 in recognition of his research into the structure of the sugar molecule; Dr. Samuel Edward Sheppard, assistant director of the research department of the Eastman Kodak Company, who was medallist in 1930, for his work in the chemistry of photography, and Dr. John Arthur Wilson, of Milwaukee, honored in 1931 for achievement in colloid chemistry, applied particularly to leather and sanitation.

Professor Conant, who was born in Boston in 1893, is a graduate of Harvard University, where he received the A.B. in 1913 and the Ph.D. in 1916. He served during the war as lieutenant in the Sanitary Corps, and later became major in the research division of the Chemical Warfare Service. An assistant professor of chemistry at Harvard after the close of the war, he became associate professor in 1925, and full professor in 1927. He is a former chairman of the organic division of the American Chemical Society. He is the author of "Organic Chemistry," joint author of "Practical Chemistry," and editor-in-chief of Volumes II and IX of "Organic Syntheses." His research has included work in reduction and oxidation, hemoglobin, free radicals, a quantitative study of organic reactions, besides the chemistry of chlorophyll.

SCIENTIFIC NOTES AND NEWS

APPOINTMENT of Dr. Vannevar Bush as vice-president of the Massachusetts Institute of Technology was announced by President Karl T. Compton following the regular meeting of the corporation on March 9. He has been a member of the faculty of electrical engineering since 1923. Dr. Bush was also elected a member of the corporation and will be dean of engineering. Plans have been made for the subdivision into the School of Science, the School of Engineering, the School of Architecture, the Division of Humanities

and the Division of Industrial Cooperation. Dr. Samuel C. Prescott, head of the department of biology and public health, will be dean of science, and Professor William Emerson, head of the department of architecture, will be dean of architecture.

DR. THOMAS HOWELL, superintendent of the New York Hospital since 1909, has been appointed assistant director of the New York Hospital-Cornell Medical College Association. The association is the coordinating agency which will open the new medical center of the two institutions, between Sixty-eighth and Seventy-first Streets, along the East River, on September 1. Dr. Howell will have charge of the general hospital activities, particularly of financial management.

DR. ROLAND THAXTER, emeritus professor of cryptogamic botany, and honorary curator of the Farlow Herbarium at Harvard University, has been elected a corresponding member of the Bavarian Academy of Sciences in Munich.

M. ACHARD, professor of clinical medicine at Paris, and Dr. Rohmer, professor of pediatrics at Strasbourg, have been elected foreign corresponding members of the Belgian Royal Academy of Medicine.

MR. VERNON BAILEY, of the U. S. Biological Survey, was one of eight honored at the first presentation of the award of The Silver Beaver of the Boy Scouts of America, consisting of diploma and medal for "distinguished service to boyhood," conferred by the organization's national council upon the recommendation of the District of Columbia executive board. The citation was "Mr. Bailey, senior biologist of the U. S. Biological Survey, became a scoutmaster in 1912 and has continued with the scout movement in various leadership capacities ever since. He has made a large contribution to scouting, developing interest in biology through the troop and camp museum, in nature understanding and outdoor lore."

THE New York Endocrinological Society was organized by a group of physicians at the Town Hall Club, New York City, on January 29, and had its second meeting, February 26, at the same place. The following officers were elected: Dr. Walter Timme, *president;* Dr. Freeman Ward, *vice-president,* and Dr. Louis Berman, *secretary-treasurer.*

AT the annual general meeting of the British Association of Economic Biologists held on February 26, the following were elected officers and council for 1932: *President,* Dr. W. B. Brierley; *Vice-presidents,* Dr. W. R. Thompson, Mr. A. D. Cotton; *Honorary Treasurer,* Dr. J. Henderson Smith; *Honorary Editors,* Dr. W. B. Brierley, Mr. D. Ward Cutler; *Honorary Secretaries,* Professor J. W. Munro, Professor W. Brown; *Council,* Mr. W. Buddin, Mr. A. D. Cotton, Mr. C. T. Gimingham, Mr. K. St. J. Cartwright, Mr. A. M. Massee, Dr. J. N. Oldham, Mr. R. C. Woodward, Dr. R. N. Chrystal, Dr. W. R. Thompson, Dr. M. A. Tincker, Dr. W. M. Ware and Mr. E. R. Speyer.

DR. JOHN J. KINDRED, a specialist in mental diseases and formerly for ten years member of the U. S. House of Representatives, has been appointed professor of medical jurisprudence in the law department of Stetson University, DeLand, Florida.

DR. HERMANN REIN, professor of physiology in the University of Freiburg in Breisgau, has been called to Göttingen.

THE Council of the University of Melbourne has passed a resolution expressing to Mr. F. Chapman, Commonwealth paleontologist, on his retirement from the position of part-time lecturer in paleontology after twelve years' service, its thanks for the help which by his wide knowledge and great experience of paleontology he has rendered to the geological department of the university.

MR. WILLIAM H. WHITE has been appointed leader of the division of truck-crop and garden insects in the Bureau of Entomology. He had been acting in charge of the division since Mr. J. E. Graf resigned in March, 1931, to become associate director of the U. S. National Museum. Mr. White has been associated with the bureau for more than fourteen years.

MR. INMAN F. ELDREDGE, for sixteen years in the Forest Service and for the last six years forest manager of a 200,000 acre private forest property in southeastern Georgia, on which he has been developing intensive forest management, has been appointed by the Forest Service director of the forest survey in the south, which is part of a nation-wide survey.

MR. D. J. MAHONY has been appointed director of the National Museum, Melbourne, Australia, in succession to James A. Kershaw, who retired last year.

MR. EMANUEL FRITZ, associate professor of forestry, University of California, has been reelected editor-in-chief of the *Journal of Forestry,* the official organ of the Society of American Foresters.

THE Sixth International Botanical Congress will be held at Amsterdam from September 9 to 14, 1935. An executive committee has been formed, the president of which is Professor Dr. F. A. F. C. Went (Utrecht), while Professor Dr. J. C. Schoute (Groningen) will act as vice-president, Dr. W. C. de Leeuw (Bilthoven) as treasurer and Dr. M. J. Sirks (Wageningen) as secretary.

DR. SAMUEL H. WILLIAMS, professor of zoology at

the University of Pittsburgh, has been appointed a member of the Pennsylvania State Game Commission by Governor Pinchot.

SIR ROY LISTER ROBINSON, technical commissioner in the British Forestry Commission, has been appointed chairman of the commission, to succeed Sir John Stirling-Maxwell, who will retire this month.

THE Massey Scientific Research Fellowship of the value of £400 per annum, recently established at University College, Nottingham, for the purpose of promoting research on cancer by physical and chemical methods, has been awarded to Mr. L. A. Woodward.

IN addition to the grants recorded in SCIENCE last week the Committee on Scientific Research of the American Medical Association has given a grant to Dr. D. W. Bronk, professor of biophysics in the School of Medicine, University of Pennsylvania, to aid him in his work on the nervous regulation of the circulation. Dr. Solomon Rosokoff with Wilson D. Langley, of the department of biological chemistry of the Medical School of the University of Buffalo, have received a grant to be used for the study of ketosis in the white rat.

DR. JOHN T. GAMBLE, professor of biology at Thiel College, has been selected as visiting professor of zoology at the University of Pittsburgh Lake Laboratory for the summer of 1932. Dr. Gamble takes the place of Professor Samuel H. Williams, associate director of the Lake Laboratory, who has been granted leave of absence.

PROFESSOR GEORGE C. EMBODY, professor of entomology in the New York State College of Agriculture at Cornell University, has left on a mission to California to study the problem of replenishing the game fisheries of that state in conference with Dr. J. O. Snyder, formerly of Stanford University, and Mr. John R. Farley, of the California Fish and Game Commission.

DR. ESMOND R. LONG, professor of pathology, University of Chicago, delivered the annual lectures in pathology at the Ohio State University College of Medicine, on February 26. The first lecture was given before the faculty and student body on "A Survey of the Origins of Modern Pathology" and the second to the faculty, the students and the medical profession of Columbus on "The Exudative Phenomena in Tuberculosis."

DR. FREDERICK H. GETMAN, of Stamford, Connecticut, lectured on the Joseph H. Johnson Foundation at Pomona College, Claremont, California, during the month of February, giving five lectures on "Electrode Potentials" and two lectures on "Color Photography."

DR. A. F. BLAKESLEE, of the department of genetics of the Carnegie Institution of Washington, lectured on February 16 before the students of the science departments of Barnard College on "Taste Worlds We Live In."

DR. CARL J. WIGGERS, head of the department of physiology of the School of Medicine of Western Reserve University, Cleveland, sailed from New York, March 10, by way of Panama Canal, for San Francisco, where he will address the meeting of the American College of Physicians on April 6. His subject will be "Arterial Sclerosis and Hypertension."

THE forty-fourth annual meeting of the American Physiological Society will be held at the University of Pennsylvania from April 27 to 30. The first scientific session will be held on the morning of April 28, the previous day being devoted to informal meetings, council meetings and visits to the various medical schools and public institutions.

THE American Society of Agronomy and the American Society of Plant Physiologists will hold a joint meeting on July 11, 12 and 13 at the Wisconsin College of Agriculture.

THE centenary meeting of the British Medical Association, under the presidency of Dr. William George Willoughby, of Eastbourne, will be held in London, beginning with the annual representative meeting on July 21. The annual general meeting will be held on July 25, and the incoming president, Lord Dawson of Penn, will give his inaugural address on July 26. The scientific sessions will be held from July 27 to 29. The centenary dinner of the association will take place July 28. There is to be a pilgrimage to Worcester on Sunday, July 24, to unveil memorials to Sir Charles Hastings, founder of the association.

THE Woman's Medical College of Pennsylvania held an open scientific meeting at the college building, March 11, marking the eighty-second anniversary of its founding. Dr. Edward B. Meigs, Washington, D. C., was the speaker of the evening on "The Nutritive Value of Milk."

THE Society of Chemical Industry has formed a food group among the members of the society. For the present, at least, there is no additional subscription beyond the usual dues for membership in the society. Further information will be furnished on request to Foster D. Snell, honorary secretary of the American Section, 130 Clinton Street, Brooklyn, New York.

Nature reports that in view of the present situation, and the recent appointment of Dr. C. P. Blacker as general secretary of the Eugenics Society, the sal-

aried appointment of Mrs. C. B. S. Hodson will terminate next August. Mrs. Hodson is to continue to work in close association with the society as a member of the council, and so secure the continuation of the eugenic work already begun in different parts of Great Britain. This arrangement leaves unaffected Mrs. Hodson's position as honorary administrative secretary to the International Federation of Eugenic Organizations.

THE lighting research laboratory of the General Electric Company and the department of research in physiological optics of the Dartmouth Medical School were awarded gold medals on March 8 by the Distinguished Service Foundation of Optometry for separate outstanding achievements in eye research. Dr. Karl Compton, president of the Massachusetts Institute of Technology, presented the medals on behalf of the foundation. The awards were made as a part of the convention of the New England Council of Optometrists. Investigations by Dr. M. Luckiesh, director of the Nela Research Laboratory at Cleveland, and his colleague, Frank K. Moss, related to the part proper illumination plays in conserving human eyesight and resulted in the creation of a new science of seeing, a combination of lighting and optics scientifically applied. The Dartmouth department was awarded a medal for its discovery of a hitherto unrecognized type of eye defect, due to small differences in the size and shape of ocular images, and the development of apparatus and lenses to detect and remedy such defect. The previously unrecognized type of eye defect was found to be an unsuspected cause of headaches, eye strain and various systemic disturbances not relieved by any retractive correction.

THE Storrow Fellowships, under the division of geology and geography of the National Research Council, are to be continued during the coming year through a personal gift of funds to Mr. Arthur Keith. The fellowships are intended primarily to assist students of promise in fitting themselves for a career of research in geology or geography, and particularly in the later stages of their preparation which might otherwise be beyond their reach. Applications for fellowships should be addressed to Mr. Arthur Keith, U. S. Geological Survey, Washington, D. C., who is chairman of the fellowship committee, and should reach him on or before the end of March. Letters in support of an application should also be furnished by persons who are acquainted with the character, record or plans of the applicant.

THE main laboratory of the Dominion Biological Station was destroyed by fire of undetermined origin on March 9. Valuable equipment and records were housed in the laboratory, which was located in Brandy Cove, near Joe's Point, a considerable distance from the main section of St. Andrews. Its value was estimated at $80,000.

ACCORDING to an Associated Press dispatch the Senate moved on March 10 to pave the way for acceptance of a $4,000,000 gift from Mr. John D. Rockefeller, Jr., for roads in the Acadia National Park in Maine by adding to the Interior Department appropriation bill $250,000 for moving the Otter Cliffs Naval Radio Station, which now blocks the route. The amendment was sponsored by Senator Porter Hale, Republican, of Maine, who announced that Mr. Rockefeller had offered the sum.

SCIENCE SERVICE reports that Delegate to Congress, Victor Houston, of Hawaii, has called attention to the fact that a reduction of $11,000 in the appropriation for the volcanology work in Hawaii and Alaska in the Geological Survey of the Interior Department will mean the dismissal of an associate topographic and geodetic engineer and an assistant geologist, who were added to the pay roll in 1932, also two local observers or field assistants in Alaska who look after the seismographs there. T. A. Jaggar, volcanologist in charge of the work in these two outposts of the United States, wrote that the increase of funds in 1932 from $21,000 to $32,000, allowed a complete reorganization of both the work and the staff, so that now "five higher officers are resident here (Hawaii), four with their own homes, and the janitor and mechanic in addition." Under present plans, two of these higher officers will have to give up their jobs.

ONE of the first shells that fell in Shanghai destroyed maps and charts compiled by Dr. George B. Cressey, professor of geology at Syracuse University, who spent seven years and traveled 30,000 miles in Manchuria and in twenty-four of the twenty-eight provinces of China, gathering material for a geological survey. The documents were stored in the building of the Commercial Press.

THE directors of the Textile Foundation, Washington, D. C., recently made an allocation of $100,000 to be spent during the course of the next two years for fellowships and scholarships in scientific research for the benefit of the textile industries. These fellowships are: Senior fellowships. For those who hold a doctorate degree or who have had equivalent training. The normal stipend is, unmarried, first appointment, $2,000; reappointment, $2,200. Married, first appointment, $2,400; reappointment, $2,700. Junior fellowships. For those who hold a bachelor's degree or who have had equivalent training. The normal stipend is, unmarried, $1,000; married, $1,200. Scholarships. Available to seniors in good standing. The normal stipend is $750. In addition to the sti-

pend, customary tuition and laboratory fees will be paid by the foundation. Conditional upon satisfactory service, the term of the senior or junior fellowship is for twelve months subject to renewal. Thirty days vacation will be allowed. Conditional upon satisfactory service, the term of the scholarship is for the academic year. All communications should be addressed to Edward T. Pickard, Secretary, The Textile Foundation, Commerce Building, Washington, D. C.

THE State University of New Jersey, now operated in conjunction with Rutgers College, at New Brunswick, is the ultimate beneficiary of the bulk of a $128,000 estate left by Edward Randolph Wood, lawyer and business man, who died on February 14 in his ninety-second year. Mrs. Wood receives the income from the estate during her lifetime. Mr. Wood suggested that it be devoted "to the study of the elimination and destruction of animal and vegetable pests." Red Oaks, the Wood summer home at Richland, N. J., near Vineland, is to be held "as a residential, social and educational center or home" for officers, faculty members, students or others connected with the university.

THE annual exhibition of current developments and activities in the field of mechanical and electrical engineering given jointly by the Yale Branch of the American Institute of Electrical Engineers and the Yale Branch of the American Society of Mechanical Engineers was held on March 7. The exhibition, staged entirely by students in the Sheffield Scientific School, gave a comprehensive survey of the part played by the mechanical and electrical engineer in modern civilization. Many of the exhibits were so arranged that the internal operation of the apparatus could be seen. The use and working principle of new machinery was explained by students; public utilities, such as a gas manufacturing plant, was shown working in miniature. Various movements in the development of machinery was illustrated by models; industrial and remote control systems were in operation in various parts of the exposition demonstrating their uses and conveniences. All the exhibits were in operation.

THE will of the late Dr. William H. Nichols has been appraised, and it is announced that half the residuary estate (less certain legacies) bequeathed to New York University, amounts to $3,670,401. Other bequests, amounting to $795,000, include $250,000 to the Polytechnic Institute, Brooklyn, and $50,000 to the American Chemical Society.

DISCUSSION

THE CONTROL OF INJURIOUS ANIMALS

UNDER the caption "The Control of Predatory Mammals," Mr. H. E. Anthony[1] expresses strong disapproval of the injurious-animal control activities of the Biological Survey, U. S. Department of Agriculture. He refers to organized propaganda carried on for several years by a small group, obviously seeking to discredit and obstruct the work.

Some of the opponents of the injurious-animal control policy of the survey are men of high attainment in their professions. Their sincerity and motives are beyond question. Most amazing to me, therefore, has been the apparent willingness of men, who I supposed were trained in the application of the scientific method, to accept as factual evidence misleading half truths and irresponsible criticisms. The attack, with its emotional appeal, is based mainly upon misdirected sentiment and distorted concepts of wild life in relation to human welfare. Its unscientific basis is shown by the absence of evidence that the value of injurious-animal control has been given any serious consideration by the opposition.

Mr. Anthony mentions the appointment by the American Society of Mammalogists at the New York meeting of May, 1930, of a committee, of which he is chairman, on the problems of predatory-animal control, instructed, he states, "to attempt a critical investigation of actual conditions in the field." The report of the committee as read by him at the Philadelphia meeting of May, 1931, consists of sweeping denunciation of the predatory-animal control work of the survey, without presenting any definite evidence obtained either by members of the committee or by its field investigators upon which such drastic conclusions could properly be based. There was an exchange of field reports, and since Anthony has used parts of survey reports that suited his purpose, it is only fair to quote from that of the only committee member who undertook extensive field studies.

Dr. C. T. Vorhies, an experienced field naturalist of the University of Arizona, designated to study field conditions in Arizona, New Mexico and Texas, approached the subject in a scientific spirit and submitted a report of 140 pages, embracing a fair review of the situation. His report was mildly critical of some features of the survey's control work and highly commendatory of others. He says:

> . . . after my own enlightening experience of the past several weeks, I may say frankly that I sincerely wish that all other members might have had the opportunity I have had for field observation *in this particular line* [italics his]. After more than twenty years in the West, and a considerable experience in the field, this was an eye opener for me! One's personal insight into the whole of the problem is so enlarged (perhaps modified)

[1] SCIENCE, 74: pp. 288–290, Sept. 18, 1931.

that I suspect that other members of the Committee will find difficulty in understanding my views and report. For example, one may readily believe from merely reading the reports of the large numbers of animals killed by the Survey that extermination of certain forms will soon result. But weeks of travel over this territory, observation of its great extent compared with the small areas actually worked at any one time or in any one year, and of the actual presence of a plentiful supply of these animals is not only enlightening but reassuring. I am deeply concerned that this committee shall not too readily accept and adopt irresponsible criticisms as their own, through lack of pertinent information, or through lack of actual contact with field conditions of this problem.

Other passages from the report of Vorhies show broad grasp of the practical considerations involved and are favorable to the survey, but are ignored by the committee's report.

Passing from predatory animals to injurious rodents, Mr. Anthony reechoes the criticism of others of the use of thallium as a poison based on statements by Dr. Jean M. Linsdale, in *The Condor*.[2] Special Publication No. 109, "The California Ground Squirrel Control Program," issued late in 1931 by the California State Department of Agriculture, presents the other side of the thallium situation and the results of an investigation of Linsdale's "cases." The cases seem to consist largely of unverified reports, which, accepted as facts, prove on investigation to be largely misrepresentations that led to sweeping and erroneous conclusions. According to the state publication, some of the alleged "cases" of thallium poisoning are from areas in which no thallium has ever been used.

Dr. Joseph Grinnell, in an editorial in the same number of *The Condor* (pp. 131–132), indorses Linsdale's "findings," and adds some speculations of his own. The publication of the California Department of Agriculture (*op. cit.*, p. 18) refers to Grinnell's editorial and comments as follows:

> It carries the implication that one third of the area of California is being repeatedly poisoned with thallium, when as a matter of fact, a survey recently completed by the State Department of Agriculture shows that less than 5 per cent of the area of California has ever been treated with thallium grain. . . . The supplementary use of thallium is largely rendering retreatment unnecessary and remarkably reducing the amount of poison used on definite areas.

Assuming the rôle of a statistician, Grinnell indulges in a process of calculation too devious for me to follow, when, apparently multiplying dead animals found by 10,000, he says: "We can figure from this that in the last four years not less than 50 *million* animals *other than* ground squirrels [italics his] have been killed in California through these operations!" One of the conclusions reached by the state publication (*op. cit.*, p. 20) is that "The *Condor* article and editorial, designed to arouse bird lovers, conservationists and the general public against the continuance of necessary pest control work, is replete with misleading information and contains few 'facts concerning the use of thallium.'"

Bubonic plague in California ground squirrels, occasionally assuming the pneumonic form, has become endemic in 14 counties in California. Human cases occur at intervals and new foci of infection are found by the Public Health Service from time to time. In the great plague epidemics of the past, the rat carriers apparently became immune, as the disease receded, after sweeping periodically across Europe, to its original home in Asia. In California, however, it is established in a group of ground squirrels (*Citellus*) of wide distribution, which with our prairie dogs (*Cynomys*) and woodchucks (*Marmota*) are closely allied to Asiatic rodents believed to be natural enzootic hosts. This alone is a sinister phase of the California situation that is ignored by propagandists against the use of thallium, a poison that, after many years of experiments, has afforded the only practicable method of controlling these animals.

The need for control of ground squirrels in California has been well set forth by the same Dr. Grinnell, who now inveighs against the use of poison.[3] Passages relating to the California ground squirrel are quoted (beginning p. 604) as follows:

> A few years ago it came into prominence as a proven disseminator of the dreaded bubonic plague, and it has become notorious for its exceeding destructiveness to cultivated crops.

Then on page 706:

> Ground squirrels breed upon cultivated or waste land, from which they invade the cultivated fields within reach as well as such other lands as are not already fully populated.

On page 704:

> On open range and pasture lands these squirrels feed largely on alfilaria and bur clover, two of the most valuable forage plants in the state. The squirrels are then serious competitors for subsistence against the flocks and herds upon which man depends for his own support. On cultivated ground these squirrels feed upon or destroy in other ways grain and fruit crops to a very large extent where present even in numbers not above those reached on wild land. *The tendency seems to be to increase to*

[2] Vol. 33, no. 3, pp. 92–106, May–June, 1931.

[3] "California Ground Squirrels," by Grinnell and Dixon, Monthly Bulletin, California State Commission of Horticulture, vol. 7, pp. 597–708, Nov.–Dec., 1918.

extraordinary numbers on cultivated lands unless effectively checked by man. [Italics mine.]

And finally, page 707:

It is hoped the facts and inferences set forth will convince the reader that the problem is not a simple one [referring to ground squirrels], and can not be solved by casual, half-hearted measures.

Contrast the foregoing with some of Grinnell's *Condor* editorial expressions (*op. cit.*), as:

There is a certain administrative type of mind to which the human "use" of all natural resources and the correlated elimination of anything which looks to be detrimental, or even not immediately and clearly of value, loom as the only "practical" aims.

And:

In our mind, at the present moment, the wholesale poisoning of wild animal life (birds, carnivorous mammals, rodents) on uncultivated terrain, ought to cease; not only that, but it should be prohibited by law.

In 1918 Grinnell pointed to ground squirrels on uncultivated land as a source of invasion of cultivated fields, and now he would prohibit poisoning them there by law. In such a view, obviously, not only the public health but economic considerations are to be entirely ignored.

The principal organizer of the campaign against effective injurious-animal control operations appears to be Mr. A. Brazier Howell, whose wide-spread propaganda and narrow view-point, plausibly presented, have undoubtedly misled many. An example of his methods in arousing prejudice against the work is his assertion, founded on the fact that certain carnivores and rodents eat grasshoppers, that he can predict outbreaks of these insects by the course of injurious-animal control operations.

Mr. Howell[4] elaborates on theories that seem to be based on his lack of understanding of wild-life conditions and the use of poison. He says:

I venture to state that it is universally believed by biologists that as rodents are now being virtually exterminated over large areas by means of poison, their places will be taken by other, and possibly more destructive, forms of life. . . . Under modern methods of poisoning, the mortality of rodents may approach 100 per cent. . . . On the surface, then, it seems that all rodents and all carnivores are gone, and everything should be lovely.

These quotations and the context show that Howell bases his absurd grasshopper predictions and other contentions on the theory that the injurious-animal control work nearly exterminates all rodents and carnivores. He has elsewhere referred to the "broadcasting of poison bait," apparently assuming that poison for rodents, at least, is regularly distributed in that way instead, as is really the case, of being placed at the holes where it is known to be consumed mainly by the animals for which intended. Many acres of unpoisoned ground, well populated by rodents of many kinds, commonly separate the holes of the injurious species where poison is placed. Any scientific investigation will reveal that the general rodent population is little disturbed by such poison operations. Upon such erroneous and misleading premises Howell bases his case against effective control of injurious species, and asks the country to accept his conclusions.

For those who do not have to bear the burden of responsibility in the solution of wild-life problems, often almost baffling in their complexity, it is easy to criticize. Some criticism must be expected and some may be deserved, but when criticism comes from professional zoologists it should be fair and made only with broad knowledge tempered with appreciation of all the difficulties that may be involved. Charges of any kind should be based upon definite scientific evidence, and such evidence has been singularly lacking throughout the obstructive campaign in progress. In my judgment one of the greatest handicaps to the real conservation of wild life in America to-day is the lack of harmony and concerted effort that results when individuals or groups who may be sincere, but misinformed and misguided, becloud issues and adopt a captious and dictatorial attitude toward those charged with carrying on wild-life administrative work.

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[4] SCIENCE, vol. 74, p. 632, Dec. 18, 1931.

THE GASTRIC EROSION OF METAL

DR. C. T. HURST, who recently[1] reported a case of gastric erosion of a fishbook swallowed by a fish, concluded that it may have taken about a year to reduce the former metal to a mere filament. A rapid gastric erosion of pieces of steel, iron or aluminum was sometimes observed in rabbits and guinea-pigs during a study of the rate of passage of inert materials through the digestive tract,[2] but the precise amount of metal dissolved was not then determined. At present, in an attempt to analyze the mechanism of the production of peptic ulcers in rats by diets low in protein,[3] a study is in progress in which the amount of metal dissolved (weight lost) is being determined in the belief that it serves as an index of gastric

[1] SCIENCE, Nov. 20.
[2] F. Hoelzel, *Amer. Jour. Physiol.*, 92, 466, 1930.
[3] F. Hoelzel and Esther Da Costa, *Proc. Soc. Exper. Biol. and Med.*, 29, 382, 1932.

acidity. The results obtained thus far mainly tend to emphasize the relation between the length of stay of the metal in the digestive tract and the degree of erosion. Thus, 100 small pieces of aluminum, which generally pass out of the stomach rapidly, have been given from time to time to rats without showing any loss in weight. Pieces of iron and steel, which remain longer in the stomach than aluminum, lost from about 0.1 per cent. (average of 200—1/16 inch stainless steel ball-bearings) to over 5.0 per cent. (average of 100 pieces of No. 18 gauge soft iron rod). Those pieces of iron and steel that remained longest in the stomach (up to 10 days) obviously lost much more weight than the average. A factor that also enters here is that, after the metal has been roughened by the initial erosion, further erosion proceeds at a faster rate. In one rat that was given a large amount of gold and silver in addition to some aluminum, a few pieces of aluminum rod (No. 20 gauge) remained about 3 days in the stomach and were eroded to the breaking point. At the usual gastric acidity of 0.5 per cent., or less, of HCl, aluminum resists erosion more than some kinds of steel and much more than soft iron. Hence, one might expect a fishhook to erode quite completely in a few weeks. Either these observations on mammals are not applicable to fish or Dr. Hurst's allowance of a year's time makes his report a typical fish story.[4]

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POLYDACTYLISM IN MICE

IN a strain of mice which carried factors for posterior duplication Danforth, in the *American Journal of Anatomy,* v. 45, 2, 1930, recorded the occurrence of extra toes on a number of animals. His investigations did not show any indication that the factor which governed the polydactylous manifestations was in any way distinct from that which governed the more pronounced effects (double feet, double legs and finally double posterior halves of the body). Rebaud, in Paris, 1919, reported polydactylous animals in a stock of mice which had an abnormal luxation of the posterior feet. The manner of the inheritance was not determined.

From a study of the literature which deals with visible characters in mice these are the only reports which have come to my attention that record the inheritance of polydactylism in stocks other than those which had been previously subjected to experimental treatment with roentgen rays.

Within the last few months polydactylism has expressed itself in a six-toed condition of the posterior feet in thirty-seven animals of a highly inbred strain of control mice in these laboratories.

The strain from which the polydactylism has arisen, as reported in SCIENCE (1931, 73, p. 482), recently underwent a mutation in hair color from chocolate brown to "leaden." At the time of the color mutation only one six-toed animal had ever been observed in the stock. This polydactylous individual was in the direct line with that of the color mutation but left no polydactylous progeny.

Among animals of the last few generations, however, the polydactylism has occurred eight times in the original stock mice, twenty-three times in pure stock mutants, and six times in the progeny from outcrosses between mutants and four unrelated strains in which the six-toed character has never been observed.

Polydactylism has been studied rather extensively in humans, guinea-pigs and poultry, and from these observations the condition is regarded as being inherited as a dominant character, expression of the character being controlled by certain unknown modifying factors. This nucleus of inbred mice offers a new species on which to investigate the method of inheritance of the character. From the preliminary matings between polydactylous mice it has already been shown that the six-toed condition does not breed as a simple recessive.

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ROSCOE B. JACKSON MEMORIAL
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TOXICITY OF SODIUM NITRATE FOR A SPECIES OF MOSS

IN an extensive series of experiments in Hampshire and Worcester Counties, Massachusetts, in which upland permanent pastures were top-dressed with Chilean sodium nitrate and other fertilizer materials, it was observed in 1929 and further confirmed in 1930 and 1931 that the nitrate was toxic to *Polytrichum commune,* a species of moss. This moss is common on "run-out" upland pastures of the New England states, the amount of moss present apparently being inversely proportional to the amount of available plant nutrients in the soil. The nitrate was used in amounts equivalent to 30, 60 and 90 pounds of nitrogen per acre. Toxicity was not as severe with 30 pounds of nitrogen in the form of nitrate as it was with 60 and 90 pounds. No definite evidence of direct toxicity from the use of limestone, hydrated lime, 16 per cent. superphosphate or muriate of potash was observed in 1930.

In 1931 a new experiment was begun in which were used the following materials: (1) Chilean nitrate of soda; (2) Arcadian nitrate of soda; (3) calcium

[4] M. Dobreff, *Pflüger's Archiv,* 217, 221, 1927, reports the finding of as high as 0.69 per cent. free HCl in the stomach of sharks.

itrate; (4) by-product ammonium sulfate; (5) calum cyanamid; (6) urea; (7) potassium nitrate; 8) potassium chloride; (9) sodium chloride. These aterials were applied to an area infested with *Polyrichum commune* at the rate of 30 and 60 pounds of itrogen per acre in case of the nitrogen carriers, and e chlorides were used in chemically equivalent mounts. Application was in the spring. Observaons made at the close of the season showed the highst degree of toxicity from sodium and potassium itrates used in the higher amounts, followed by the odium and potassium chlorides in chemically equivaent amounts and by sodium and potassium nitrates the smaller rates. The other nitrogen carriers, ncluding calcium nitrate, showed no toxic effects.

From observations thus far made it appears that oxicity of certain nitrates for this species of moss eems to be due primarily to the sodium and potassium ons. However, the cationic effect seems to be linked omewhat with the anionic effect, for, with one exception the nitrates were more toxic than the corresponding chlorides. This observed toxicity is not an indirect ffect, that is, a crowding out of moss by other vegetation, as was first supposed, but is an actual killing f the moss. The effect is immediate and in proportion to the amount of sodium nitrate applied. It is lso cumulative, so that after several smaller applications the effect is similar to that of a single larger pplication.

A. B. BEAUMONT

MASSACHUSETTS AGRICULTURAL EXPERIMENT STATION

PREHISTORIC MOUNDS IN SOUTH FLORIDA

SEVERAL professors connected with the University of Miami have been working on a mound recently discovered on Key Largo which is suspiciously Mayan in character. It is a stone structure carefully constructed by aboriginal masons and is strikingly like several structures in British Honduras. The character of the pottery found in the neighborhood indicates a foreign origin, since there is no pottery clay in Southern Florida. It is black and hard like the pottery of Yucatan. Obsidian knives and other remains discovered in the vicinity of the mound are also indicative of foreign influences. Various canals and small harbors dug in this region indicate that Southwestern Florida was once inhabited by a numerous and enterprising population. The Mayans were great seamen and traders and it is more than likely that they settled in Florida. The abundance of game, especially fish and shell-fish, would have been a great attraction. There is reason for believing that the Calusas were of Mayan stock and that even the Seminole may have Calusa blood in his veins. This region will be included in the proposed National Everglades Park and it is hoped the Seminoles may be used for guards and guides.

The University of Miami, under whose auspices the preliminary researches have been made, will continue its investigations in this region and will later publish fully the results of its studies.

JOHN C. GIFFORD
ALFRED H. GILBERT

CORAL GABLES, FLORIDA

SCIENTIFIC BOOKS

Comité national français de Géodésie et Géophysique. Assemblée générale du 9 mai 1931. Au Secrétariat général du Comité. Rue d'Anjou, 78, Paris 8e.

THIS publication gives the proceedings of the 1931 annual meeting of the French National Committee on Geodesy and Geophysics. These proceedings cover only some nine pages. The remainder of the volume of over 90 pages is occupied with reports of the sections dealing with the various special branches of geophysics and with membership lists. The reports themselves are summaries, or sometimes summaries of summaries, so it would be rather absurd to carry the process of summarization still farther in this review. The reviewer will therefore confine himself to making a few general remarks, based on this publication as a text, and to mentioning a few of the items found merely because they happen to be connected with fields of work in which he is especially interested or to strike his fancy as odd or noteworthy. A different reviewer would no doubt find texts for different sermons and would single out different items for special mention.

It is instructive to read publications of this sort. They bring before the reader the great diversity and the wide ramifications of geophysics and, in spite of these, its essential unity. The subjects treated in the different sectional reports vary greatly, but the membership of the sections overlaps extensively (just as it does in the case of the American Geophysical Union), and everywhere problems are encountered that concern more than one section. Geophysics extends on one side into geology and geography, on the other sides into technical physics and astronomy. If any one is to be immune from the dangers of too narrow specialization, the geophysicist ought to be.

The meeting of the French National Committee looked both to the immediate past and the near future.

Much space was given in all sectional reports to the meeting of the International Geodetic and Geophysical Union that had been held at Stockholm the year before; and much space also, especially in some sectional reports, to the plans for the International Polar year of 1932–33.

It is interesting—at least to a geodesist—to note that the French section of geodesy has the largest membership of all the sections (nearly the reverse is true of the American Geophysical Union). Not all the members of the section are professional geodesists. Perhaps the explanation is that the place of geodesy in general geophysics is something like that of anatomy in the medical sciences; that is, geodesy like anatomy, is a fundamental subject, some knowledge of which is required by the specialist in other subjects.

In the Section of Geodesy considerable attention was paid to gravity at sea. It was hoped that Dr. F. A. Vening Meinesz, of Holland, who has perfected a method for obtaining accurate values of gravity at sea by means of a specially constructed apparatus used aboard an immersed submarine, would be admitted to a French submarine to take observations and to train others in the art. At the last moment, however, these plans had to be cancelled because of objection on the part of the Naval General Staff. It may be added, however, that since the date of the meeting here reported, the work of obtaining gravity at sea has made notable progress. Many observations of gravity over the Mediterranean have been made with Meinesz's apparatus in an Italian submarine; Meinesz himself is at this writing (February, 1932) engaged in his second gravimetric campaign in an American submarine operating among the West Indies and the Bahamas; and only Meinesz's departure for the United States to take part in this campaign prevented his acting as instructor to a French hydrographer and a French naval officer who were to be sent to Holland to be trained by him. It is therefore to be supposed that eventually the French navy will participate in this important work.

Mr. Holweck and Father Lejay have developed an apparatus for the rapid determination of gravity, of which great things are hoped. In principle it is a metallic strip clamped to point upward and to execute elastic vibrations. The vibrations are greatly slowed down by means of a load applied to the strip. The slowing down varies with the intensity of gravity, hence the period of vibration under the combined effects of the elasticity and of the load may be used to determine relative gravity. The crucial question is: will the elastic properties of the metal remain sufficiently constant with age and use?

The Section of Seismology reports plans for a first-class earthquake observatory at Martinique. More observatories like this are needed in this unstable region and its nearness to Mont Pelé will add to its value. The Union of Socialist Soviet Republics in its Stockholm report tells of very extensive plans for seismological study. If fully carried out, they will add much to our knowledge.

One more item in the report of the Section of Seismology deserves mention, though it is of a quite different order of magnitude, so to speak, from the comprehensive plans of the Soviet Government. At Le Mans (in Maine, France, not Maine, U. S. A.) there was a seismological station maintained by the energies of one man and the support of the community. This one man, Mr. Jagot, did an excellent work not only in maintaining the station but in arousing popular interest in the subject and in spreading a knowledge of it. Now, being 77 years old, he feels compelled to retire. It would be a fine thing if competent amateurs in science were everywhere more numerous and were also interested in spreading a knowledge of science. Seismological institutions also might well consider whether they should not give more attention to popularizing their subject.

The Section of Seismology also reports that for lack of radio apparatus many French seismological stations are unable to take advantage of the seismological broadcasts sent out from time to time from the U. S. Naval Station at Arlington along with the daily weather forecast. By way of explanation it may be added that these messages result from international cooperation and from the collaboration of stations in the United States, Canada and the Pacific area, of the Coast and Geodetic Survey, of the Jesuit Seismological Association and of Science Service.

The report of the Section of Oceanography mentions the possibility of adapting the Favé tide-gauge, which is a pressure gauge for use at sea, to the study of surface waves, adding that experimental work on these short-period surface waves is much needed. The report also mentions the formation of an international committee to study the so-called (and miscalled) tidal waves (in French "raz de marée)." Presumably these "tidal waves" are solitary waves or small groups of waves caused by submarine earthquakes. Some less misleading term than "tidal wave" is needed and the noncommittal Japanese word *tsunami* has been suggested but has so far found no general acceptance. (This comment on nomenclature is the reviewer's, not the report's). In the report of the same section are some interesting remarks on the fallibility of water temperatures as an indication of the nearness of icebergs.

The Section of Hydrology reports a test made to

check the operations of a magician, presumably—from the context—a finder of water by means of a divining rod. As was to be expected, the so-called magic was found to yield no results of value. The section is reported to be on the lookout for further tests of this sort in order to clear away superstition and increase real knowledge. This raises the question of the duty of scientific organizations when they encounter superstitious beliefs in their special fields. Should they sit back and do nothing, on the theory that a superstitious man is immune to reason and that attempts to argue him out of his superstition are pure waste of energy, or should every opportunity be sought not only to diffuse knowledge but also to combat superstition?

Two difficulties are more than once referred to that are familiar enough to geophysicists on this side of the Atlantic also. One is the difficulty in getting money for geophysical work; the other is the unwillingness of organizations engaged in commercial geophysical work to make their results public in order that general scientific knowledge may be advanced thereby.

The above are samples of the nuggets that may be found even by a hasty perusal of what at first glance would seem to be an exceedingly dry routine report.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

A HOLDER FOR CHICKENS AND OTHER BIRDS

IN an effort to find a practical means for holding chickens without the aid of an assistant a board was designed which can be used for confining fowls quickly and firmly in either ventral, dorsal or lateral positions.

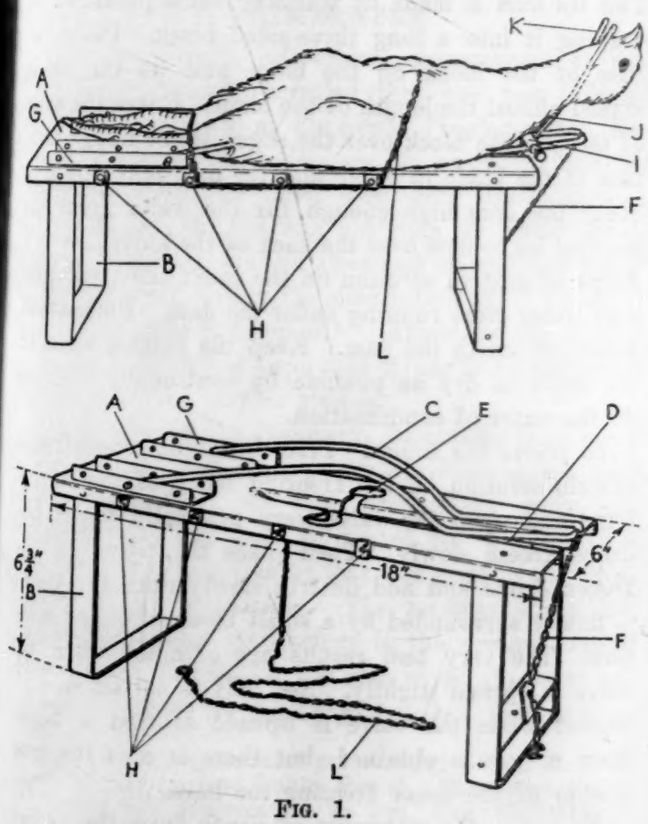

FIG. 1.

This board (Fig. 1) consists of a sheet of metal supported on legs to form a low table and a loop of metal, curved to conform somewhat to the shape

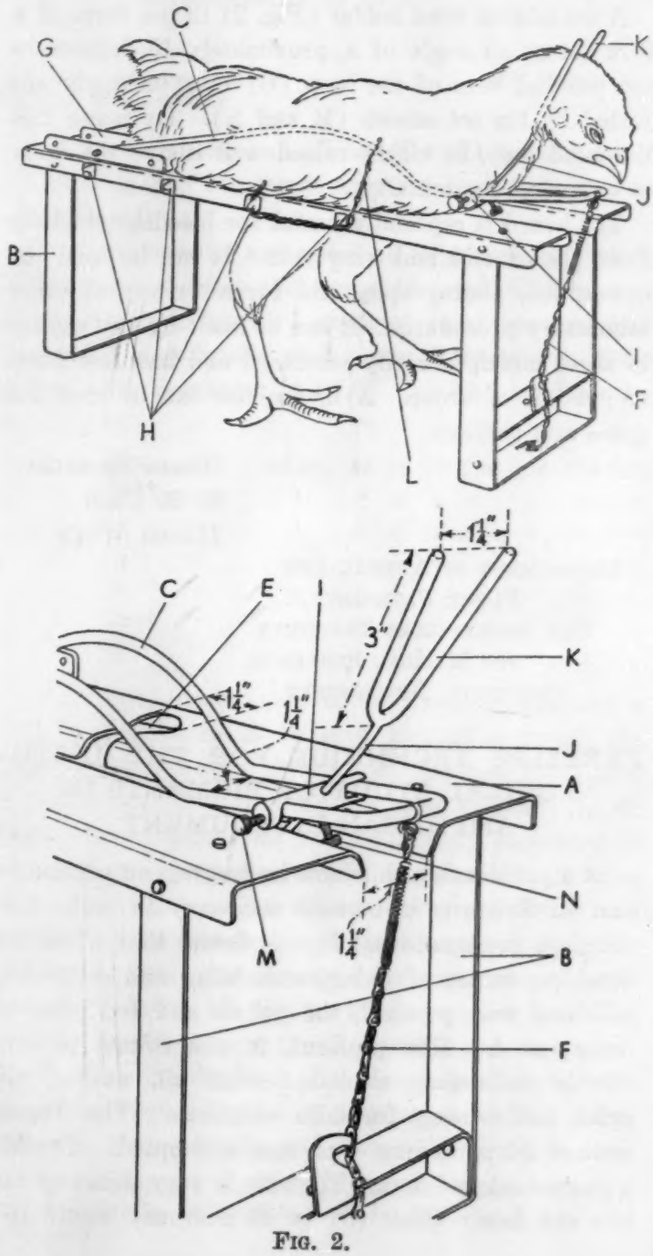

FIG. 2.

of the chicken's body, hinged to one end (C). A long notch (D), 1¼" across, cut lengthwise in the board with two circular perforations (E) located on either side and joined to it, provides a place for the legs and sternum when the chicken is in the ventral position, and for the vertebral column and ilium when in the dorsal position. The loop (C), which can be attached at various points (G) depending on the size of the chicken, fits over the body in either the dorsal or ventral position and holds it firmly against the board. It is fastened with the chain and spring (F). Another chain (L) holds the legs when a chicken is fixed in the dorsal position and may be used with the loop or alone for fastening the body to the board. It is particularly useful for restraining a chicken in either the dorsal or lateral position with the wings through the notch (D).

A detachable head holder (Fig. 2) in the form of a fork set at an angle of approximately 45 degrees to the parallel bars of the loop (C) may be easily adjusted by the set screws (M and N). By using this the head may be either raised well above the body or extended horizontally.

The board is especially useful for bleeding chickens from the carotid and wing vein. It can be used for operations, photography and for a variety of other laboratory procedures. It can be made by any worker in sheet metal, is easily sterilized and has few parts to get out of order. A larger size can be used for geese and turkeys.

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FREEZING TECHNIQUE FOR THE HISTOLOGICAL STUDY OF PIGMENTS IN AMPHIBIAN INTEGUMENT

IN a problem which is now in progress on pigmentation in Triturus it became necessary to make histological preparations. It was found that when the usual procedure of fixing, embedding and sectioning of tissue was pursued, the yellow and red pigment disappeared. This pigment, it was found, is very soluble in strong alcohols, cedar oil, aniline oil, xylol and strong formalin solutions. The frozen method of sectioning was then attempted. Trouble was encountered here. The skin is very thin and unless cut fairly thick (30 to 40 microns) would roll and wrinkle to such an extent during the process of washing and staining that the section would be made useless. This was remedied by placing the tissue in a weak solution of formalin for a half an hour to an hour. Too strong a solution of, or too long a time in formalin would cause the pigment to be dissolved. But a half hour in 10 per cent. formalin was sufficient and gave the desired rigidity to the tissue.

Other disturbing factors were that, first, when making sections in warm weather, the blade would melt the frozen block while cutting the tissue; and secondly, it was difficult to get the tissue off the blade before it melted. There was always great danger of damaging the tissue when taking it from the blade in the melted condition. These disturbing factors were remedied by our "ice dam."

The entire procedure for sectioning and staining is given below:

Place the fresh tissue in a 10 per cent. solution of formalin for half an hour to an hour.

Wash the tissue in water for an hour after taking it from formalin. Then place in a concentrated solution of dextrin for three to twelve hours.

Build on the microtome an "ice dam" of paraffin. This ice dam is made by warming some paraffin and shaping it into a long three-sided block. Place the base of the block on the blade and let the block extend almost the length of the blade. Curve the ends of the paraffin block over the edges of the upper portion of the blade in order that the dam may not slip. Make the dam high enough for the water from the melting ice to flow over the back of the knife. A few drops of dextrin solution on the inner edge will prevent water from running under the dam. Place small blocks of ice in the dam. Keep the cutting edge of the knife as dry as possible by continually blotting off the water of condensation.

To freeze the tissue: Place drops of concentrated dextrin solution on the freezing block of the microtome and open the valve very gradually to let the drops freeze slowly. Then place the tissue on the frozen drops and add dextrin slowly until the tissue is finally surrounded by a small block of sugar solution. The very best results are obtained when the valve is opened slightly. Not only is carbon dioxide wasted when the valve is opened so that a heavy blow of gas is obtained, but there is also the possibility of the tissue freezing too hard.

To obtain the maximum efficiency from the carbon dioxide, place the tank with the nozzle end at the level of the microtome and the base of the tank at least a foot higher than the nozzle level.

As soon as the tissue is frozen, begin sectioning. Hold the handle firmly in the right hand and pull the

knife across the block quickly. With a camel's hair brush in the left hand, quickly remove the section from the knife before it melts. If the section melts, one is likely to damage the tissue in brushing it from the blade.

Place the section in a syracuse dish of Ringer's or physiological salt solution. When a sufficient number of sections have been made, they may be stained.

By means of a small glass rod, headed on the end like a balsam dropper, remove the section from the solution. Let the section wrap around the beaded portion; it has been hardened enough in formalin to hold its shape fairly well. The section may be held up slightly in the solution by means of a dissecting needle while the glass rod is being slipped under it. With the section remaining on the glass rod, dip it into the stain for ten seconds (the stain I found best for this tissue was polychrome methylene blue). From the stain place the section into Ringer's or physiological salt solution. It is all right to let the section drift in this solution, for the stain gives it added rigidity so that it may easily be picked up again with the rod.

Now, still by means of the glass rod, transfer the section to a slide on which is a drop of Brun's Glucose Medium. By rolling the rod through the drop of liquid the section can be made readily to come off of it. (Glycerine alone takes the stain from the tissue.) A cover slip may now be placed over the tissue. This must be done carefully, for the tissue is often wrinkled in the placing of the cover slip.

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SPECIAL ARTICLES

THE CULTIVATION OF A SPECIES OF TROGLODYTELLA, A LARGE CILIATE, FROM THE CHIMPANZEE

THE examination of several chimpanzees at this laboratory recently revealed that one was infected with a large ciliate of the genus *Troglodytella*. Reports of such an infection are not numerous and the organism seems to have been very little studied. Although this species has not been definitely determined it has been studied alive and stained and found to answer very closely the description of *T. brassarti* var. *accuminata* Reichenow.[2,3] Attention was at once directed to it in the hope that it might be cultivated and be used for further studies.

At the time of the discovery, *Balantidia* from other individuals of the same group of chimpanzees were being cultivated. In this preliminary work the *Troglodytella* has been cultivated for a short time following the same technic that was used in the cultivation of the *Balantidia*. The medium which has proven to be most satisfactory in the writer's experience for the *Balantidia* has also given the most encouraging results for the *Troglodytella*. This medium is that of Tanabe and Chiba[4] for the cultivation of *E. histolytica*. Pig serum only has been used in these experiments.

Greatest success was obtained in an experiment in which six test tubes containing 10 cc of solution were inoculated with about 1 gram of fecal material containing *Troglodytella* and a few *Balantidia*. These tubes were incubated at 37.5° C. The results were as follows:

Tubes	Serum	24 hrs.	48	72	96	120	144	168	172
1	5 per cent.	+	+	+	+	+	+	+	−
2	5 " "	+	+	+	+	+	+	+	−
3	5 " "	+	+	+	−				
4	10 " "	+	+	+	+	+	+	+	−
5	10 " "	+	+	+	−				
6	10 " "	+	+	+	−				

In 24 hours many very active *Troglodytella* and a few *Balantidia* could be seen swimming in all the tubes. Subcultures were made at this time from tube 4, which seemed to contain the most ciliates. In 48 hours the *Troglodytella* had apparently increased in numbers and a number of dividing individuals were seen at this time. The ciliates were very active and some could be seen boring into the agar slant. The *Balantidia* had also increased in numbers. At the end of 96 hours all ciliates in three tubes had died and in the other three the *Balantidia* had multiplied much more rapidly than the *Troglodytella* and outnumbered them. The latter, however, were still active and both *Troglodytella* and *Balantidia* could be seen

[1] From the department of protozoology, Johns Hopkins School of Hygiene and Public Health. The writer wishes to express his appreciation to Drs. Van Volkenburgh and Long and the Committee on Cold Research, from whose chimpanzee the material for this work was secured.

[2] E. Reichenow, "Den Wiederkäuerinfusorien verwandte Formen aus Gorilla und Schimpanse," *Arch. f. Prot.*, 41: 1–33, 1920.

[3] J. Buisson, "Les infusoires ciliés du tube digestif de l'homme et des mammifères," Trav. Lab. Parasit. Fac. Méd., Paris, 1923.

[4] M. Tanabe and E. Chiba, "A New Culture Medium for *Endamoeba histolytica*," *Acta Med. in Keijo*, 11: 1–4, 1928.

boring into the slant. At about this time unfavorable conditions set in in the tubes and multiplication of both ciliates seemed to cease, and at the end of the seventh day, all of both types were dead.

Five tubes were inoculated for the first subculture. Only two of these were positive at the end of 48 hours. In these two the *Troglodytella* seemed to have multiplied considerably at the end of 48 hours and dividing individuals could be seen. The *Balantidia* multiplied much more rapidly, however, than the *Troglodytella* and at the end of 72 hours far outnumbered them. Both types remained active until the end of the sixth day when they all died. Second subcultures were made at the end of 48 hours. In these, twenty-four hours later, the *Balantidia* outnumbered the *Troglodytella*, and, although the latter were alive at the end of 96 hours when subcultures were again made, only the *Balantidia* survived this transfer.

This experiment was repeated several times with the same result and then several other media[5,6,7] were tried but without success. It is possible that the failure of continued cultivation of the ciliate may not have been due so much to any fault of the medium as to the fact that such frequently repeated transfers does not give time for sufficient multiplication as it does in the case of the *Balantidia*, a smaller ciliate. This problem is being worked on at the present time in this laboratory.

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THE EXPERIMENTAL TRANSMISSION OF ANAPLASMOSIS BY DERMACENTOR VARIABILIS

ABOUT a year ago the experimental transmission of bovine anaplasmosis by the brown dog tick, *Rhipicephalus sanguineus*, was reported by the writer[1] from this laboratory. This tick, although very widely distributed throughout all tropical countries of the world, is only known to occur in Texas, Louisiana, Mississippi, Florida, Kansas, Ohio, Pennsylvania and New York so far as this country is concerned, whereas anaplasmosis occurs in an area which, while not yet well mapped, certainly extends not only beyond the zones within which the related disease, piroplasmosis (cattle tick fever), is now confined, but also outside the original range of enzootic bovine piroplasmosis. It was pointed out, therefore, that if anaplasmosis is tick-borne throughout its entire range, it should have, judging from the distribution of ticks in the United States, at least two other species of ticks as its carriers. The experimental evidence presented in the present paper incriminates, as a carrier of anaplasmosis, another tick, *Dermacentor variabilis*, a species important because of its wide distribution and host range. This tick occurs on the Pacific coast and appears to be wide-spread east of a line extending south from the middle of the Canadian border of North Dakota to about Corpus Christi, Texas. It is not known to be present in the Rocky Mountain States. There is grave danger, therefore, that this tick may carry this destructive disease into many areas that hitherto have been considered outside the enzootic range of anaplasmosis.

EXPERIMENTAL CONDITIONS

Since not only the adults but also the larvae and the nymphs of three-host ticks drop to the ground after engorging, it is necessary in experimental work to devise means of retrieving the engorged ticks so that they will not be lost in the stable litter. When available, therefore, bulls have been used in preference to cows since the ticks could be held in a bag which was attached by means of adhesive tape to the bull's scrotum. For the experiments reported in the present paper, the bulls were purchased late in the fall in an area in Colorado where nothing resembling anaplasmosis is known to occur and were shipped immediately to Jeanerette, Louisiana, where they were confined in a screened barn. A description of this barn in which the bulls were kept out of contact with biting flies and other ectoparasites except the ticks used in the experiments has been published by the writer.[1] Bulls that have been held in this barn and not yet used for experiments were checks on the work; during the work herein reported five such checks were held and all of them remained uninfected. Furthermore, during the past three years, 68 head of cattle have been held in this barn, some of them as long as 18 months; except when experimentally transmitted, not a single case of anaplasmosis has been noted.

EXPERIMENTAL PROCEDURE

Many unengorged and partly engorged adults of *Dermacentor variabilis* were taken from cattle in Mississippi and Florida during April and May, 1931, by tick inspectors under the direction of Dr. Hartwell Robbins, of the U. S. Bureau of Animal Industry, and by Dr. T. W. Cole, of the U. S. Bureau of Animal Industry. These ticks were sent alive to the laboratory at Jeanerette, Louisiana, where they were used as follows: (1) Adults were allowed to engorge on a bull with clinical anaplasmosis, the females

[5] E. Schumaker, "The Cultivation of *Balantidium coli*," *The Amer. Jour. Hyg.*, 13: 1, 281–295, 1931.

[6] Sidney Margolin, "Methods for the Cultivation of Cattle Ciliates," *Biol. Bull.*, 59: 3, 1930.

[7] A. Schourenkova and V. Nossine, "Le culture du *Balantidium coli l'ovigen humaine*," La presse medicale, 10: p. 1686, 1930.

[1] C. W. Rees, "The Experimental Transmission of Anaplasmosis by *Rhipicephalus sanguineus*," *North American Veterinarian*, September, 1930.

viposited in the laboratory, and the larvae engorged two susceptible bulls; (2) the larvae engorged on clinical case of anaplasmosis and the nymphs from ese larvae engorged on two susceptible bulls; and 3) the nymphs engorged on a clinical case of naplasmosis and the adults from these nymphs engorged on two susceptible bulls. In this way anaplasosis was transmitted to the last four of the six sceptible bulls, transmission occurring in the moves om larvae to nymph and from nymph to adult. ince the first two bulls have not thus far reacted, e larvae did not "inherit" the infection from their others, *i.e.*, the etiological agent of anaplasmosis did ot persist in the eggs of the ticks in the step from dult to larva, as is the case with the etiological agent f bovine piroplasmosis.

Test of Hereditary Transmission

On May 20, 1931, some unengorged adults of *ermacentor variabilis* were permitted to engorge on ull No. 47, and on May 25–26 seven engorged feales were removed. Bull No. 47, having been plenectomized on May 13, had clinical anaplasmosis uring the time of this engorgement. The severity f this attack was demonstrated by the fact that on ay 26, 375 erythrocytes per 1,000 were found to e infected with *Anaplasma*. The female ticks viposited in the laboratory, and from June 30 to uly 8 several thousand larvae engorged on a susptible bull, No. 71. Several thousand other larvae rom these females engorged from July 13 to July 20 n another susceptible bull, No. 68. As stated above either of these bulls has reacted to anaplasmosis. heir susceptibility has not yet been proved by the injection of virulent blood. The blood of bull No. 7 has, however, been proved to be capable of transitting anaplasmosis by injecting about 40 cc of it n May 25 into a susceptible bull No. 53. No. 53 reacted to anaplasmosis on June 9 and died on June 12.

Larva to Nymph Transmission

Many of the partly engorged female ticks oviposited n the laboratory without further engorgement; other emales were permitted to engorge on rabbits. From he eggs of both lots of these females many larvae were hatched, and on July 7 the larvae were placed in bag which was attached to bull No. 65. Bull No. 65 ad very severe clinical anaplasmosis at this time. He had been inoculated intravenously on June 16 with virulent blood from bull 46 and reacted to naplasmosis on July 7. On July 13 a count showed hat 370 erythrocytes per 1,000 were infected with *naplasma*. A blood count on July 17 showed only ,140,000 erythrocytes per cubic millimeter and there were 19,600 white blood cells.

The larvae engorged until July 14 and molted in the laboratory to nymphs which were divided into two lots. On July 29 one lot was placed on a susceptible bull, No. 62. However, on August 5 no engorged nymphs could be found on this bull, though many remained unengorged in the bag. On this date other nymphs were added to those already in the bag and the latter was reattached to the scrotum. These latter nymphs were from another lot of larvae which had engorged from June 28 to July 5 on bull No. 46. Bull No. 46 failed to react to anaplasmosis after blood injection on April 13. He was, therefore, splenectomized on June 16 and a mild case of anaplasmosis was diagnosed on June 29. There was only a slight rise in temperature and the *Anaplasma* were never numerous but were present in the smears for about six weeks. The virulence of the blood of this bull No. 46 was proven because as stated above he was the "donor" of the blood used on June 16 to inject into bull No. 65.

Only 14 engorged nymphs were removed (August 10 to August 15) from bull No. 62, but many were found to have been crushed in the bag. Bull No. 62 reacted to anaplasmosis on September 2. The incubation period was, therefore, not longer than 35 days.

On August 4, the other lot of nymphs mentioned above as from the larvae that engorged on bull No. 65 were placed on bull No. 67. Thirty of them were removed, engorged, from August 9 to August 15. Bull No. 67 reacted to anaplasmosis on September 4. The incubation period, therefore, was not longer than 32 days. Both bulls No. 62 and No. 67 had irregular temperatures, fluctuating between 102° F. and 106° F., until about September 15. Anaplasma were numerous, as were also the characteristics of severe anemia. On September 12 the blood counts were as follows: Bull No. 62, erythrocytes 2,460,000, leucocytes 14,600 per cu mm; bull No. 67, erythrocytes 1,460,000, leucocytes 14,300 per cu mm. However, neither bull lost his appetite nor had other discernible symptoms of severe illness. The infectivity of the blood of these bulls has not yet been tested by injection into susceptible cattle.

Nymph to Adult Transmission

Other nymphs of *Dermacentor variabilis* were secured by engorging the larvae on rabbits. On July 14 several hundred of these nymphs were placed on bull No. 65. Seventy-six of them engorged and were removed on July 20 to 22. Bull No. 65 was convalescing from anaplasmosis during this time, having reacted, as noted above, on July 7. He was still weak and very anemic and occasional *Anaplasma* could still be found in the smears. The engorged

nymphs molted in the laboratory to adults and on August 10 about half of them were placed on bull No. 73, the other half on bull No. 74. Between August 17 and August 21, five engorged females were removed from No. 73 and 9 from No. 74. A number of males, not counted, were also removed from each bull. Bull No. 73 reacted to anaplasmosis on September 13, and bull No. 74 on September 14. The incubation periods were, therefore, not more than 34 and 35 days, respectively.

DISCUSSION

The data of the present paper appear to warrant the following general statements:

1. Larvae of *Dermacentor variabilis* may acquire anaplasmosis by engorging on a carrier, *i.e.*, a bovine in which the blood carries the etiological agent of this disease, and nymphs which develop from these larvae may transmit anaplasmosis to susceptible bovines.

2. Nymphs may become infected by engorging on a carrier, and adults which develop from these nymphs may transmit anaplasmosis to susceptible bovines.

In the present experiments the adult female ticks which engorged on carriers did not transmit anaplasmosis to the larvae of the next generation. More experimental work is needed, however, to determine whether or not hereditary transmission by this tick may occur. Had the experiment been tried, the nymphs or the adults of the second generation might have transmitted the infection, even though the larvae failed to do so. Furthermore, it may be possible for females to become infected either from larvae or nymphs rather than from engorging directly on a carrier and to transmit the disease to the larvae, nymphs or adults of the next generation.

It is possible, though not at all probable, that the nymphs and the adults which were used with success in the experiments of the present paper "inherited" their infections from the adults which were sent from Mississippi and from Florida. Be that as it may, the important feature of this work is that *Dermacentor variabilis*, which is one of the most widely distributed ticks in the United States, will serve in the transmission of anaplasmosis. This discovery warrants the serious attention of all those engaged in dairying or in the production of beef cattle because, as stated above, this destructive disease may be spreading at the present time into new areas.

It has been found that ticks of several genera can and do transmit anaplasmosis in various parts of the world, and with this evidence to the effect that the etiological agent of the disease has so little specificity in its requirements for an intermediate host one is safe in assuming that other ticks, not yet known to transmit anaplasmosis in the United States, actually do transmit it. Certain ones of these ticks, if in criminated, would suffice to make the known distribution of anaplasmosis coincide with the known distribution of carriers.

It is of interest to note that *D. variabilis*, convicted here of carrying anaplasmosis of cattle, has recently been incriminated as a carrier of a disease indistinguishable from Rocky Mountain spotted fever in man. These findings bring this tick to the front as a parasite of major importance.

SUMMARY

Under properly checked and controlled conditions the writer has succeeded in transmitting anaplasmosis by means of *Dermacentor variabilis* as follows: (1) Ticks engorging as larvae on clinical cases of anaplasmosis transmitted the disease as nymphs to two susceptible bulls; (2) ticks engorging as nymphs on a convalescing case of anaplasmosis transmitted the infection to two susceptible bulls.

The test of "hereditary" transmission was negative when the ticks engorged as adults on a clinical case and the larvae of the next generation engorged on two susceptible bulls.

The wide-spread occurrence of *Dermacentor variabilis* in the United States indicates the danger that anaplasmosis may spread into new areas that have thus far been considered as outside the enzootic range of this disease.

The recent incrimination of *Dermacentor variabilis* in the transmission of what appears to be Rocky Mountain spotted fever has focused the attention of the medical profession and of public health officials on this tick, and this finding and the writer's findings indicate the need of further research on it as a tick of major importance in human and veterinary medicine.

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